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EPIDEMIOLOGICAL NOTES AND REPORTS

PENTACHLOROPHENOL POISONING IN NEWBORN INFANTS – St. Louis, Missouri

From April to August 1967, nine cases of a clinically distinct illness characterized by fever and profuse sweating occurred in a small nursery for newborns in St. Louis, Missouri. Two of the cases were fatal. Early in the course of the outbreak the disease was felt to be an intoxication, but the nature of the poison and the mode of exposure of the patients remained obscure. Only after the ninth case developed was it discovered that an antimildew agent, containing a high concentration of sodium pentachlorophenate (the sodium salt of pentachlorophenol), was being used in the hospital laundry. All of the clinical, epidemiological, and biochemical evidence indicated that this outbreak resulted from pentachlorophenol poisoning. The only identified mode of exposure was skin absorption of sodium pentachlorophenate residues on diapers and other fabrics, resulting from the misuse of the antimildew agent in the final laundry rinse.

The outstanding clinical feature of the illness was extreme diaphoresis. Attendants consistently noticed that the infants' clothing and brows were drenched with sweat. Nevertheless, the neonates nursed avidly. As the disease progressed, fever rose as high as 103°F, respiratory rates increased, and breathing became labored, though auscultation of the lungs was normal and cyanosis was absent. Other common findings included tachycardia, hepatomegaly, and irritability followed by lethargy. Anorexia, vomiting, and diarrhea were notably absent. Stiffness of the neck, muscular fasciculations, and convulsions were not observed. Skin rashes or evidences of inflammation or irritation of the skin were not seen.

Laboratory tests frequently showed a progressive metabolic acidosis, proteinuria, a rising blood urea nitrogen, and "pneumonia" or "bronchiolitis" on X-ray. Bacterial and viral cultures of blood, cerebrospinal fluid, nose, throat and stool revealed no pathogens. Autopsy findings of the two fatal cases showed fatty metamorphosis of the liver in both cases and fatty vacuolar changes in the renal tubules of one case.

All except one of the seriously ill infants, a fatal case, were transferred to other hospitals for treatment. After the first fatal case occurred, the attending physicians suspected a toxic cause and therefore promptly performed exchange transfusions on each of the seriously ill infants who were subsequently transferred for medical care. This treatment yielded dramatic results. Within minutes to hours, the infants became more responsive and had less respiratory distress. Fever and sweating disappeared, as did metabolic acidosis. Renal function returned to normal during the next few days. Except for the two fatal cases, recovery was apparently complete.

The first four cases developed between April 17 and 19 among a group of 25 infants who were in the nursery during this interval. The first infant to become ill died. The institution was closed on April 24 and thoroughly cleaned and disinfected before re-opening on May 3. A second cluster of four cases occurred between May 10 and 15. One of these also was fatal. The average age of these eight cases, at onset of illness, was 8.9 days. Several additional suspect cases with fever and sweating were detected among 13 infants who had been discharged from the hospital in apparent health between April 17 and May 15.

From the time of the first recognition of the outbreak, an intensive and persistent search was made for toxic substances in the environment of the infants. A solid-stick evaporating deodorizer had been used without change in practice for 4 years. A commercial exterminator had sprayed regularly with a carbonate insecticide monthly for 2 years within the hospital, but never in the nursery. The management of drugs and the preparation of babies' formulas revealed no deviations that were likely to permit the introduction of a toxic substance to this many babies.

For the preceding 10 months, a commercially available disinfectant containing a mixture of synthetic phenolic derivatives had been used extensively and frequently in the nursery, and had been repeatedly applied to surfaces that came in contact with infants' skin.

One-dimensional thin-layer chromatography of serum specimens obtained from the first eight cases was performed. These tests revealed the presence of a phenolic substance in all test specimens, which was similar to a phenolic ingredient of the disinfectant. This substance was thought to be the toxic chemical causing the disease.

The nursery was closed and recleaned. Use of the suspect germicidal detergent was abandoned, and all equipment that had been treated with it was discarded or rendered free of phenolic residues by extensive cleaning with alcohol. New linens and diapers were purchased and the nursery reopened July 11.

On August 29, an 8-day-old infant had the acute onset of an illness identical to the previous eight infants. The infant received an exchange transfusion and promptly recovered. A follow-up survey of infants discharged from the hospital in July and August revealed six additional infants who had the characteristic excessive sweating in a milder form of the same syndrome.

The formerly suspect disinfectant was no longer in use. Reinvestigation of laundry procedures disclosed a previously overlooked source of phenols. An antimildew agent, containing 22.9 percent sodium pentachlorophenate and 4.0 percent trichlorocarbanilide, was being used in the terminal rinse of all nursery linens and diapers, despite a warning on the label that the compound "must not be used" in laundering diapers.

This product had been in use in the laundry since March 1966. The recommended quantity was one ounce of powder per laundering cycle, but it was ascertained that the laundry was actually using 3 to 4 ounces.

Thin-layer chromatography of the serum and urine of the new case revealed an abnormal substance with characteristics that were identical to those detected in the pre-

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vious infants' sera. Further studies in two different laboratories with improved methods of analysis have shown that the chemical in the urine and serum of the new case was pentachlorophenol, and was clearly not one of the phenolic ingredients in the previously suspected disinfectant. Additionally, pentachlorophenol was identified in freshly laundered diapers obtained from the nursery. The quantity of pentachlorophenol varied from 1.5 to 5.7 mg per diaper. Unfortunately, no sera from the earlier cases remained for these more sophisticated analyses.

Actions have been instituted to prevent further illnesses that might be caused by the misuse of this product, or two other sodium pentachlorophenate-containing products that are recommended for similar purposes. The manufacturer has been directed to trace all sales and shipments of these products during the past 18 months, and to remove such products from all hospitals and any establishment that is involved in general laundry work. The company has voluntarily ceased sale of these three products.

(Reported by J. Earl Smith, M.D., Health Commissioner, Division of Health, Department of Health and Hospitals, City of St. Louis, Missouri; L. E. Loveless, Ph.D., Chemist, Clinical Laboratories, St. Louis, Missouri; E. A. Belden, M.D., Consultant, Communicable Disease Control, Local Health Services Section, Division of Health, Missouri Department of Public Health and Welfare; the Epidemiology and Pesticides Programs of the National Communicable Disease Center, Atlanta, Georgia; the Toxicology Section, Occupational Health Program, National Center for Urban and Industrial Health, Cincinnati, Ohio; the Pesticides Regulation Division, Agricultural Research Service, United States Department of Agriculture, Washington, D.C.; and a team of EIS Officers.) .

Editorial Note:

The clinical, laboratory, epidemiological, and pathological findings, as well as the prompt response to exchange transfusion, all indicate a toxic, rather than an infectious, cause of this outbreak. The fever, sweating, and acidosis are consistent with intoxication with certain phenolic derivatives, which are known to increase the metabolic rate[1]. The symptoms described here are remarkably similar to industrial accidental poisonings resulting from overexposure to pentachlorophenol or its sodium salts[2,3]. The exact manner in which the infants became poisoned cannot be established, but the most reasonable explanation is absorption through the intact skin as a result of repeated contact with diapers, blankets, and linens containing small, but readily absorbable, quantities of sodium pentachlorophenate. The antimildew agent, which is labelled not for use in laundering diapers or hospital linens, nevertheless, was in use in this hospital. Pediatricians, hospital administrators, housekeepers, and local health authorities should check commercial diaper services and hospital laundries to ensure that this product is not in use.

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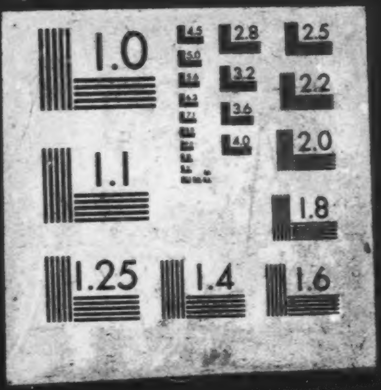
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The Effect of Aerially Applied Malathion on an Urban Population

Small

Allan L. Gardner, MD, and Ronald E. Iverson, MD, Atlanta

During the St. Louis encephalitis epidemic in Corpus Christi, Tex, in the fall of 1966, malathion was applied by aerial spraying over the city and outskirts to control the vector mosquito. A group of 119 volunteers who received varying degrees of exposure to this spray was studied. This involved comparing pre-spray and postspray cholinesterase activities and compiling histories of exposure and symptoms. A 5% incidence of mild and transient symptoms such as headache, nausea, and weakness was noted in the exposed volunteers, but there were no pathognomonic signs. There was no correlation of symptom frequency or severity with enzyme activity and no statistically or clinically significant change in enzyme activity related to time of spraying. It was concluded that there is negligible risk to human health involved in aerial applications of malathion.

AN EPIDEMIC of St. Louis encephalitis (SLE) occurred in Corpus Christi, Tex, in August and September of 1966. The epidemiological investigation reported by Williams[1] revealed 223 suspected cases of SLE and demonstrated 99 cases with serological evidence of recent infection. The *Culex quinquefasciatus* mosquito was the vector.

Malathion has been shown to be effective in mosquito control.[2] Studies conducted in Florida, Puerto Rico, and South Carolina showed that three liquid ounces of active 95% malathion per acre effectively control the *Aedes aegypti* mosquito.[2] Therefore, in Corpus Christi 95% concentrate was applied aerially using this low volume technique[3] over the entire city and the immediately surrounding area (approximately 100,000 acres) by three US Air Force C 123's. Evaluation of the *C quinquefasciatus* population by Public Health Service entomologists indicated that this treatment provided 90% to 95% reduction in the adults and 70% to 75% reduction in larvae. The spraying occurred approximately at what proved to be the peak of the epidemic curve,[1] and after the initiation of spraying, the incidence of encephalitis cases dropped to almost zero within two weeks, which is approximately the incubation period of the SLE virus in man.[4] The natural epidemic curve for SLE in man usually has a period of decline greater than two weeks.[5,6] These facts suggest that the malathion spraying was instrumental in curbing the outbreak of SLE.

Malathion (O, O-dimethyl S-[1, 2 dicarbethyloxyethyl] phosphonodithioate), an organic phosphorous insecticide, exerts its clinical effects on humans primarily by inhibiting the enzyme cholinesterase[7] thereby increasing the concentration of acetylcholine with its subsequent ganglionic and parasympathetic postganglionic stimulation. Malathion is, however, one of the least active cholinesterase inhibitors in mammals.

Malathion has been studied extensively over the past ten years, and its safety for people has been demonstrated under a number of conditions of use by numerous authors.[8-14] Moeller et al[13,14] also demonstrated that an oral intake of 24 mg/day/man of malathion for 56 days will cause a cholinesterase depression without the occurrence of clinical symptoms. Other authors[7,11] have corroborated this finding of cholinesterase depression in the absence as well as in the presence of clinical findings. Therefore, measurement of cholinesterase is a valid test

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Table 1.—*Correlation of PEI With Frequency of Symptoms and Changes in Plasma Cholinesterase Activity*

				Mean (± SE) Plasma Cholinesterase*		
PEI	N	F	F/N	Prespray (Sample 1)	1 Day Post (Sample 2)	3 Days Post (Sample 3)
0	7	0	0	5.3 ± 0.2	5.0 ± 0.3	5.2 ± 0.4
1+	17	0	0	5.1 ± 0.2	5.0 ± 0.3	4.9 ± 0.2
2+	63	4	0.06	5.1 ± 0.1	5.1 ± 0.1	5.0 ± 0.1
3+	27	0	0	4.9 ± 0.2	5.1 ± 0.2	5.2 ± 0.2
4+	5	2	0.4	5.3 ± 0.3	4.7 ± 0.3	5.3 ± 0.1
Totals	119	6	0.05	5.1 ± 0.1	5.0 ± 0.1	5.0 ± 0.1

*Normal range is 2.9 μmol to 6.8 μmol/min/ml. N indicates number in group; F, frequency of symptoms.

for the minimal effects of the compound in man.

Malathion is safe when used with appropriate precautions, but there is risk when the precautions are not followed. This risk is demonstrated by acute poisoning cases reported in children receiving high malathion exposure.[15-18] Also, some individuals who handle malathion develop allergic manifestations such as contact dermatitis.[19]

Despite reports of malathion's low toxicity, members of the general population express fear of any proposed spraying. When a large population in Corpus Christi, Tex (approximately 200,000) was exposed to malathion by aerial spraying, this study was carried out to determine the effect on the general population of exposure to malathion under these conditions of use.

Table 2.—*Evaluation of Pre-spray and Postspray Plasma Cholinesterase in High and Low Exposure Groups**

		Matched Samples†		
PEI	N	1-2	1-3	2-3
Low (0&1+)	24	Z = 1.3‡	Z = 1	Z <1
High (3+&4+)	32	Z <1	Z = 2.2	Z <1.5

*Evaluation by Wilcoxon Matched Pair Signed Rank Test.

†Sample 1 indicates prespray; 2, one day after; 3, three days after.

‡Z <1.96 not significant at the 5% level (ie, $p>0.05$), where Z is the standard normal variable.

Materials and Methods

A group of 164 policemen and firemen volunteers, selected only by their presence at work and willingness to participate in the study, was used as a sample of the adult population exposed to the spraying operations. Varying amounts of exposure were anticipated because of differences in job location.

The individuals in the study group were seen three times for interviews and blood sampling. The initial visit was made about 24 hours prespraying, while the second and third visits were made approximately 24 hours (first day) and 72 hours (third day) postspraying. The individuals at the prespraying visit were questioned briefly about past medical history, family history, previous pesticide exposure, and current symptoms. The individuals were questioned at the second visit about the occurrence of symptoms in themselves or their families during the 24 hours following the spraying.

A general estimate of the exposure of each individual to malathion was determined at the time of the second visit. The postspray questionnaire asked the location of the participant during the spraying hours and in the 24 hours postspraying; whether he smelled the malathion; whether he saw the airplanes, and, if so, how many times the planes passed overhead; whether he saw any killed insects in the area; etc. A rough Pesticide Exposure Index (PEI) was then constructed. The sample was subgrouped into 0 exposure (ie, not in sprayed area) up to 4+ exposure (ie, outdoors and under the spray planes for several passes).

The heparinized venous blood samples taken at each visit were refrigerated and shipped on ice to the Atlanta National Communicable Disease Center (NCDC) Toxicology Laboratory. Determination of red blood cell (RBC) and plasma cholinesterase activity was carried out by the pH Stat method of Nabb et al.[20] The frequency and type of symptoms and changes in the cholinesterase activity of the population in each PEI subgroup were analyzed.

Results

Of the original 164 participants, 119 remained with the study to provide complete historical and chemical data. Because of tech-

Table 3.—*All Individuals With Symptoms and Their PEI and Cholinesterase Values*

Serial No.	PEI	Plasma Cholinesterase* µmol/min/ml			Symptoms
		Prespray	1 Day Postspray	3 Days Postspray	
12	2+	4.9	5.4	3.4	Nausea
30	2+	5.3	5.1	5.0	Headache, slight nausea
38	2+	5.7	4.5	5.4	Headache
52	2+	4.4	3.9	4.1	Headache
65	4+	4.4	4.8	5.2	Headache, nausea, weakness
152	4+	5.9	5.3	5.6	Headache, nausea

*Normal range is 2.9 µmol to 6.5 µmol/min/ml.

nical difficulties from laboratory relocation and the demonstrated decay of cholinesterase activity during two months of storage, the RBC determinations were considered invalid and discarded. This was not felt to invalidate the study because it is well known that the plasma cholinesterase, although less specific, is more sensitive to inhibition by malathion and the first enzyme to show decreased activity in man.[13]

Previous pesticide exposure either involved compounds that would not inhibit cholinesterase or had occurred too long before this study to be of any consequence. The cholinesterase values were all within the normal range of 2.9µmol to 6.8µmol/min/ml except in two cases where low values were recorded from the prespray samples. The distribution of the population over the exposure range, the frequency of symptoms, and the mean cholinesterase activity before and after spraying is shown in Table 1. The distribution fell in fairly close agreement with the binomial distribution, but the number (N) in the extreme groups is too small for statistically valid inferences to be drawn (at the 5% level). Nevertheless, it is interesting to note that there is a slight trend for symptom frequency to follow degree of exposure. The plasma cholinesterase activity means do not differ when related to PEI category or to time of spraying.

In Table 2 the extreme subgroups have been combined to yield minimally significant N's in a low (0 and 1+) and a high (3+ and 4+) PEI group which were then examined by the Wilcoxon Matched Pair Signed Rank test for significant differences in prespray and postspray cholinesterase activity. If the spraying had provided an inhibiting dose, a *decrease* in activity which should be greater in the high group than in the low would be expected. However, the data shows no differences significant at the 5% level except for the high group's first sample (prespray) compared with the third (72 hours postspray). In this instance there was an *increase* in activity, which is a change opposite to that expected from malathion.

Six subjects reported symptoms which were as follows: one with nausea, two with headaches, two with headaches and nausea, and one with headaches, nausea, and slight weakness (Table 3). Table 3 further emphasizes the lack of correlation of changes in enzyme activity with occurrence of symptoms as well as with PEI. In fact, subject 65, who had the most symptoms and was judged 4+ exposed on the PEI, showed rising activity throughout the sampling period.

None of the symptomatic people were disabled or kept from work, and all had recovered prior to the drawing of the first postspray blood. Neither these nor any other participants reported symptoms in their families starting during the study period.

Conclusions

While the symptoms elicited are consistent with malathion exposure, other causes, such as an upper-respiratory tract infection or a mild attack of the epidemic SLE virus, may have made a contribution to the observed frequency of symptoms. The symptoms of nausea, weakness, and headache are nonspecific for organophosphorous poisoning, and the lack of characteristic findings such as fasciculations and miosis should be noted. The slight trend toward correlation of PEI and symptom frequency noted above may also be accounted for by the fact that the volunteers were subject to suggestion by repetition of a specific symptom questionnaire. In the volunteers, those exposed to malathion would be more susceptible to suggestion; therefore, their symptoms may have been psychogenic. There are no data in this study which would allow distinction between the possible etiologies.

In cases of poisoning by malathion, a direct correlation is almost always present between frequency or severity of symptoms and cholinesterase activity,[7,13] and in poisoned individuals a depression in cholinesterase would be expected. However, the fact that even in the 4+ PEI group no one had the 25% to 50% decrease in enzyme activity that various authors have suggested as a criterion of excessive exposure is a good indication of how little effective dosage the human population received during this aerial application. The failure to demonstrate a significant difference in mean cholinesterase activities in prespray and postspray samples in all pesticide exposure groups further justifies the inference that the malathion spraying had no influence on the changes observed. Moreover, even assuming that all reported symptoms were due to pesticide exposure, the low symptom frequency in our sample and the mildness of the symptoms which did occur make the fears about aerial spraying of malathion seem unfounded. Thus, the potential aid in aborting the SLE epidemic far outweighs the negligible risk involved.

Joseph E. Suggs, PhD, research chemist, supervised the running of the plasma and red cell cholinesterases in the Toxicology Laboratory, NCDC. William R. Metzger, MD, Director of Public Health and Welfare, Corpus Christi—Nueces County Health Department, Corpus Christi, Tex, and his staff provided assistance in organizing study groups and interviewing individuals.

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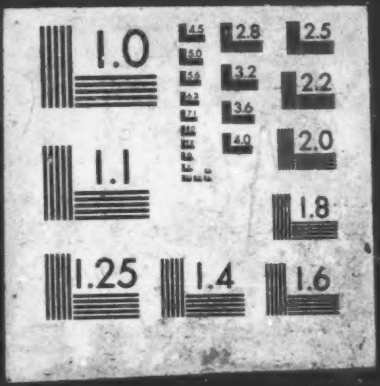
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Arsenic Levels in Urine of Forest Workers Applying Silvicides

Small

Robert F. Tarrant, Corvallis, Ore, and Jack Allard, PhD, Wenatchee, Wash

Forest tree thinning workers absorb arsenic from silvicides. Much of the chemical appears to be excreted from the body in a short time. There appears to be no evidence of a continuing increase in arsenic levels over a period of more than two months. Persons using these materials should be trained to observe all necessary precautions to minimize their exposure.

THINNING overdense stands to improve growth rate of trees is one way in which the nation's supply of merchantable timber can be increased significantly.[1] In the Pacific Northwest alone, several million acres of such dense precommercial stands would benefit from thinning.

Two basic methods are used to thin dense forest stands—felling unwanted trees with saws or killing them with chemicals. Economics aside, thinning by felling concentrates slash on the ground and presents a fire hazard, unsightly appearance, and decreased access to forage for livestock and game animals. Chemically killed trees are left standing. The leaves or needles fall first, then the branches, and eventually the stems. Chemical thinning allows better use of forage, is esthetically more appealing, and presumably is less of a fire hazard. For these and other reasons, the possibility of using chemicals to kill unwanted trees is of great interest.

Cacodylic acid (dimethylarsinic acid) and monosodium methanearsonate (MSMA) are used by public and private forestry agencies in chemical tree thinning programs throughout the Pacific Northwest and elsewhere in the United States. The chemicals are injected into the tree cambium by a variety of techniques, all of which may expose the worker to the chemicals. Since this forest management technique is used widely, the matter of human safety is of concern.

In 1968, the US Forest Service and the Washington State Department of Health, through its Community Pesticide Study project headquartered at Wenatchee, Wash, obtained preliminary data on the level of arsenic in three thinning crew workers exposed to this element. These observations indicated need for a larger, more sensitive study of the potential for arsenic intake by forest workers using arsenical silvicides. In the study reported here, total arsenic concentration, determined by arsenic levels in the urine, was related to method of application, chemical, and length of time of exposure.

Methods

Three six-man crews from the Wenatchee and Okanogan National Forests in north-cen-

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tral Washington were selected for study. Thinning procedures assigned to each of the six men in each crew were recorded in Table 1.

Crewman 1 was a Forest Service employee living and working in the same area as the thinning crew members but not working with chemicals. Crewmen 2 and 3 used the injection hatchet to apply chemical to trees. This tool automatically meters a prescribed amount of chemical into the tree cambium when the blade strikes the tree. Crewmen 4 and 5 used the hack-squirt method of applying the chemical. This involves making a hatchet cut in the tree, into which the chemical is introduced from a plastic squeeze bottle. Crewman 6 used an injector tool which, after impact with the base of the tree, is manipulated to inject chemical from a reservoir in the handle into the cambium layer.

In order to minimize variations in exposure, all study subjects were required to wear similar clothing and use the same safety practices. Every morning each crew member was supplied a set of freshly laundered outer garments consisting of trousers, a shirt, and two pairs of cotton gloves. The gloves, worn at all times while applying the chemicals, were changed at noon or when they became contaminated. Eye protection consisted of goggles or "wraparound" sunglasses. Adequate cleaning facilities were maintained in the field for each man. The study members were asked to wash their hands before smoking or eating. Crew members filled, cleaned, and generally maintained their own equipment. The chemical dispensing apparatuses were dismantled and thoroughly cleaned at least once a day. Major repairs to equipment were made by the crew foreman. Empty containers and wash water were disposed of by the study members under supervision of the foreman.

The study was carried out during the summer months over a period of nine weeks. Urine samples were collected from each worker in each crew on Monday morning before work was begun and again on the following Friday afternoon at the end of the working day.

Urine was analyzed for total arsenic content by digesting a 100-ml aliquot of sample with a nitric-sulfuric acid mixture to oxidize all organic material. The remaining solution was analyzed for total arsenic by converting any arsenates present to arsine gas, which was trapped in a solution containing silver diethyldithiocarbamate. Arsine in the presence of this carbamate forms a colored complex. The color intensity was measured, and the amount of arsenic present in the original urine sample was calculated. In lieu of 24-hour collections, urine concentration differences were corrected by use of osmolality values. The analytical method[2] proved sensitive to 0.01 ppm. Arsenic recovery ranged between 85% and 100%.

Table 1.—Thinning Procedures Assigned to Men in Each Crew

Crewman	Chemical Used	Application Method
1	None	None
2	Cacodylic acid	Injection hatchet
3	MSMA	Injection hatchet
4	Cacodylic acid	Hack-squirt
5	MSMA	Hack-squirt
6	MSMA	Injector tool

Results and Comment

Absence of workers for a variety of reasons made it difficult to obtain urine samples from every study participant at every sampling time. Data are missing in at least one instance for every week except the fourth (Table 2). However, complete data are available for weeks 2, 3, 4, 7, and 8, except for the treatment by the injection hatchet with cacodylic acid. Results of an analysis of variance performed on these complete data indicate that the arsenic content of urine of workers exposed to chemicals was significantly higher than that of workers in the control group (Table 3). There were no significant differences among the noncontrol treatments.

Concentration levels of total arsenic in urine were elevated after a week of exposure to the chemicals regardless of the method of application or chemical used. The high values observed on Friday were, in most instances, near normal again on Monday. In individual cases where a very high value (over 1 ppm) was measured on Friday, the weekend absence from contact with the chemicals did not allow sufficient time for arsenic levels to return to normal. Therefore, Monday values were relatively high in these instances.

Greatly elevated arsenic values could generally be explained as the result of unusual exposure, eg, the men may have accidently cut themselves with hatchets, spilled chemicals on their clothing and skin, had to make frequent equipment repairs, or may have become lax in their safety habits. Statistical analysis shows that arsenic concentrations in urine on Fridays were significantly greater than those measured in samples taken on Mondays (Table 3). Thus, we conclude that the generally higher values found on Friday were real and that arsenic concentration in urine will increase during any work week. However, there was no indication of a continuing increase over the nine-week study period.

Complete data were available for all treatments, including that with the injection hatchet with cacodylic acid only, on the basis of Friday observations for weeks 2, 3, 4, and 6. These data were analyzed to compare arsenic levels in urine on the basis of chemical used and on the two methods of application, hack-squirt and injection hatchet. The difference between chemicals was not statistically significant (Table 4). Neither was the difference between application methods, although with both MSMA and cacodylic acid arsenic concentration of urine of workers using the injection hatchet was substantially greater than that of those employing the hack-squirt method.

The arsenic level of urine at which concern for matters of health should begin is not well known. A manufacturer of arsenical silvicides states:

> Because some individuals are more sensitive than others, it is advisable to always be on the lookout for signs of skin sensitivity among persons handling arsenicals. When a person shows sensitivity, he should be removed from exposure. When an individual is exposed daily for extended periods, the inspection for skin sensitivity should be supplemented by monthly urine analysis for arsenic. . . . When a sample shows above about 0.3 mg of arsenic per liter, another sample should be taken and if again above this figure, the donor should be removed

Table 2.—Total Arsenic Concentration in Urine of Forest Workers Variously Exposed to Arsenical Silvicides: Means of Three Observations

Treatment	Week 1	2	3	4	5	6	7	8	9
	Total Arsenic (ppm)								
	Monday								
Control, no exposure	...*	0.03	0.05	0.01	0.02	0.03	0.07	0.02	0.07
Injector, MSMA	...	0.08	0.07	0.09	0.05	...	0.10	0.13	0.05
Hack-squirt, MSMA	0.02	0.06	0.04	0.07	0.07	0.10	0.12	0.07	...
Hack-squirt, cacodylic acid	0.02	0.09	0.06	0.06	...	0.15	0.12	0.09	0.09
Injection hatchet, MSMA	...	0.09	0.08	0.04	0.05	0.12	0.13	0.17	...
Injection hatchet, cacodylic acid	...	...	...	0.20	0.21	...	0.37	...	...
	Friday								
Control, no exposure	...	0.06	0.04	0.05	...	0.03	0.04	0.14	...
Injector, MSMA	0.18	0.35	0.24	0.58	0.25	0.34	0.56	0.07	...
Hack-squirt, MSMA	0.27	0.25	0.30	0.19	0.23	0.42	0.36	0.20	0.22
Hack-squirt, cacodylic acid	0.22	0.40	0.28	0.51	0.31	0.50	0.51	0.32	0.29
Injection hatchet, MSMA	0.35	0.93	0.26	0.49	0.24	0.54	0.28	0.53	...
Injection hatchet, cacodylic acid	...	0.64	0.76	0.50	0.75	0.88	...	...	...

* Data missing for at least one of three replications (workers).

Table 3.—Means of Three Observations of Arsenic Levels in Exposed Workers for Weeks 2, 3, 4, 7, and 8

Treatment*	Total Arsenic (ppm) Monday	Friday
Control, no exposure	0.04 ± 0.01	0.07 ± 0.03
Injector, MSMA	0.10 ± 0.01	0.36 ± 0.07
Hack-squirt, cacodylic acid	0.03 ± 0.01	0.41 ± 0.08
Hack-squirt, MSMA	0.07 ± 0.01	0.26 ± 0.05
Injection hatchet, MSMA†	0.10 ± 0.01	0.50 ± 0.12
Av of all treatments	0.08 ± 0.01	0.32 ± 0.03

* Control is significantly lower (5% level of probability) than all chemical treatments. No chemical treatment is significantly different from any other. Monday concentrations are significantly lower than those of Friday.

† Injection hatchet-cacodylic acid treatment not included in analysis because of missing data. Means (in ppm) for weeks 4 and 5 are: Monday, 0.21; Friday, 0.63.

Table 4.—Mean Total Arsenic Concentration in Exposed Workers by Chemical and Method of Application

Treatment	Mean* ± SE (ppm)
Chemicals	
MSMA	0.42 ± 0.08
Cacodylic acid	0.56 ± 0.09
Application methods	
Hack-squirt	0.36 ± 0.05
Injection hatchet	0.62 ± 0.10

* Differences between the two chemical means and between the two method means were not significant when tested at the 5% probability level.

Table 5.—Comparison of Friday and Following Monday Levels of Arsenic and Observations in Which Total Arsenic Exceeded 0.3 ppm

Treatment	Total Observations	Observations in Which Total Arsenic Exceeded 0.3 ppm: Friday	Observations in Which Total Arsenic Exceeded 0.3 ppm: Following Monday
Control, no exposure	23	1	0
Hack-squirt, MSMA	25	8	0
Injector, MSMA	24	10	0
Hack-squirt, cacodylic acid	24	11	0
Injection hatchet, cacodylic acid	18	12	5
Injection hatchet, MSMA	24	13	0

from exposure. Actual experience with . . . (persons involved in the manufacturing operation of arsenical materials) . . . shows that removal from exposure quickly reduces the urine arsenic level to normal and eliminates the signs of skin sensitivity.[3]

All but one of the 15 men applying the chemicals in this study had urine arsenic levels in excess of 0.3 ppm at least once during their period of exposure. The highest level recorded was 2.5 ppm. However, except for those workers using the injection hatchet with cacodylic acid, in all subjects in whom the Friday arsenic level exceeded 0.3 ppm, the level on the following Monday was always less than 0.3 ppm (Table 5).

No health problems were encountered in the study group that could be classified as arsenic poisoning. There were 136 man-weeks of exposure to arsenical silvicides recorded for study members. During that exposure period, 14 men complained of headaches, six of abdominal cramps, five of weakness, four of nausea, and one of skin rash. The controls, observed for 27 man-weeks, had one episode of nausea and one of weakness. The number of persons, especially controls, under observation was small; and no firm conclusions can be drawn, although the large number of reports of headache may have some significance. The crew members were aware that they were part of a health study of an arsenic compound and, as a result, may have overreported symptoms. They did not, however, have an increased rate of sick leave over other Forest Service employees.

Conclusions

Forest tree thinning workers absorb arsenic from silvicides, much of which appears to be excreted from the body in a short time. Measurements over a period of more than two months indicated there was no increase in levels of arsenic in urine after the first week. However, excretion was always greater at the end of each work week than after weekend absence from contact with the chemicals.

Most workers in this study exceeded a total arsenic concentration of 0.3 ppm in urine on one or more occasions during the study. No evaluation of the health effects of this exposure can be made from this study.

The amount of arsenic absorbed by workers could not be related to kinds of arsenical chemicals used or to methods of application.

This study indicates the possibility that tree thinning workers are exposed to excessive quantities of arsenic. Thinning crews using these materials must be trained to avoid skin contact with the chemicals. Protective clothing, including gloves of material impervious to the chemical solution, is most necessary. Water-repellent cream should be used on the face and any other skin areas that cannot otherwise be protected. Extreme care must always be used in handling chemicals, especially when filling application tools where the danger of spillage is great. Furthermore, facilities must be provided for thorough hand and face washing after periods of chemical use.

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PESTICIDE RESIDUES IN FOODS IN RELATION TO HUMAN HEALTH

BY

W. F. DURHAM

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Pesticide residues in foods in relation to human health

By
William F. Durham *

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I. Introduction

The presence in the general food or water supply of any foreign chemical is a matter not to be dismissed lightly. The pesticides are poisons, of course, or they would not be useful in the control of insects, rodents, and other undesirable animals and plants. The ingestion by the population of small amounts of these pesticidal chemicals as residues on and in food has been viewed with alarm by a number of people. It is important, however, to consider the quantitative dose-response relationships involved.

The present paper reviews the available data bearing on the hazard to human health of pesticide residues in foods.

In considering the overall result of pesticide usage on public health, one should not overlook the fact that, although some health hazards are associated with the use of these compounds, a number of health benefits have resulted also. Pesticides promote health directly through the control of insect-vector-borne diseases and indirectly through increased and improved agricultural production of food and fiber.

II. Pharmacologic responses of experimental animals and man to pesticides

a) Acute and chronic toxicity

The preliminary evaluation of chemicals which may occur in trace quantities in food is based exclusively on animals tests, analytic methods, and the results of residue measurements. The U. S. Food and Drug Administration has published a description of the tests required to obtain approval for sale of a pesticidal chemical which may leave a residue in food (Lehman *et al.* 1959). Barnes and Denz (1954) have reviewed the scientific aspects of this matter.

No attempt will be made in this review to summarize the various animal toxicity studies which have been carried out on pesticides. The reader is referred to several excellent papers on this subject including particularly the work of Lehman (1951) and of Gaines (1960). The acute LD_{50} value and the maximum tolerated repeated dose are quantities which are of value in judging the relative hazard of a specific compound. Although compounds of higher insect and mammalian toxicity are contained at lower concentrations in use formulations, in most instances this decrease in concentration is not great enough to compensate for the toxicity difference. Use experience has confirmed this impression, since workers applying formulations containing compounds of higher toxicity, particularly the more toxic organic phosphorus compounds, are poisoned more often than workers using less toxic materials. For example, in California in 1957, of a total of 252 cases of systemic poisoning attributed to agricultural chemicals, 189 cases (75 percent) were associated with exposure to organic phosphorus compounds (Kleinman *et al.* 1960).

Although there is considerable overlapping in toxicity between the groups, on the average the acute toxicity of the organic phosphorus compounds is somewhat greater than that of the chlorinated hydrocarbon materials. However, the chlorinated hydrocarbon compounds, due to their greater stability, are considerably more of a residue problem than are the organic phosphorus pesticides (DURHAM 1961).

There is a useful correlation between occupational hazard of pesticides to man based on actual use experience and toxicity levels as determined in experimental animals (GAINES 1960). GAINES has presented evidence that there is a closer relationship between dermal LD_{50} values and the occurrence of occupational poisoning than between oral LD_{50} values and occupational poisoning. The prediction of occupational poisoning may be improved somewhat by studying the effect of repeated dermal doses of a pesticide in animals (HAYES 1960). Recommendations of safe practices for workers using pesticides usually advise against eating or smoking while working with these materials. This precaution would seem reasonable; however, proved methods are not yet available for measuring primary oral exposure (DURHAM and WOLFE 1962). WOLFE *et al.* (1963) have reported that spraymen using dieldrin[5] or endrin do not contaminate food or cigarettes with significant quantities of these insecticides even when handling the food or tobacco items without washing their hands. However, QUINBY *et al.* (1963) have attributed the poisoning of a sprayman to his consumption of a candy bar which had become contaminated with parathion spray or concentrate during the work day.

b) *Special toxicity studies*

In addition to studies of morbidity and mortality, evaluation of the hazard to the general population of long-term exposure to residues in food or water must also include consideration of other measures of response of the animal or man to the toxicant. Some of these responses, including pathology, potentiation, neurotoxicity, and carcinogenic effect, are considered below.

1. Pathology. With the exception of the neurotoxic effect which occurs with certain compounds, the pathology associated with exposure to the organic phosphorus pesticides is not noteworthy. However, the chlorinated hydrocarbon compounds do produce significant histopathology, particularly of the liver, in animals which are subjected to high levels over long periods of time.

The histopathology associated with pesticides which has caused the most discussion occurs in animals given repeated doses of DDT or of certain other of the chlorinated hydrocarbon compounds. Histological changes occur in the livers of rats even at very low levels of DDT in their diet. These changes were first reported by KUNZE *et al.* (1949). These workers reported that histopathology could be detected in the livers of rats maintained for six months on a diet containing 5 p.p.m. of DDT. However, CAMERON and CHENG (1951) were unable to demonstrate any pathology in rats sacrificed after being dosed for more than a year at levels corresponding to food concentrations up to approximately 350 p.p.m. Some other workers, including DEICHMANN and colleagues (1950) and TREON and CLEVELAND (1955), reported liver changes induced by relatively low dosages of DDT, while HAAG and associates (1948) and GREENWOOD and coworkers (1953) failed to find such changes. This controversy has been reviewed in detail by HAYES (1959 b).

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[5] Well-known pesticide chemicals are identified in text by commonly accepted or trade names, with occasional alternates in parentheses; those not commonly known are identified chemically the first time used.

In 1956, ORTEGA and his co-workers published a paper summarizing an extensive series of tests with rats designed to settle the question of the occurrence of morphologic changes induced in the rodent liver by low levels of DDT. These authors found that liver cell necrosis occurred with dosages in excess of 1,000 p.p.m. but not at lower levels. Histological changes restricted to the liver occurred at levels as low as 5 p.p.m., but liver function, as measured by bromsulphthalein excretion, was not affected in rats fed 400 p.p.m. or less. The histological changes in the parenchymal cells of the liver consisted of an increased deposition of fat, margination of cytoplasmic granules, hypertrophy of the cells, and most characteristic — the formation of complex, lipoid cytoplasmic inclusion bodies termed "lipospheres". ORTEGA (1962) has recently reported additional details of these cytoplasmic alterations as noted in studies using both light and electron microscopy. These changes could be recognized in some male rats at levels of 5 or 15 p.p.m., but in females only at 200 p.p.m. Increased severity of the changes was noted with increasing dosage. The authors concluded that cellular alterations were demonstrable in the livers of rats fed DDT at dietary levels as low as 5 p.p.m. However, they considered it possible that occurrence of the changes depended not only on DDT but also on some unidentified secondary factor. Furthermore, they suggested that greater stress be placed on the mildness of the changes when they do occur, on their ready reversibility, and on their imperfect correlation with liver function or with the clinical effect of DDT. The changes under discussion seem to occur commonly in rodents only; their significance, even for rats, is far from clear.

DURHAM *et al.* (1963) were able to detect no clinical signs of illness consistent with DDT poisoning and no liver histopathology in monkeys fed DDT at dietary levels of 200 p.p.m. or less for periods of up to 7.5 years. All of six monkeys fed diets containing 5,000 p.p.m. of DDT developed tremors, convulsions, and other classical signs of DDT poisoning, but only one of these animals showed the cytoplasmic inclusions which have been characteristically associated with chlorinated hydrocarbon poisoning in the rat. Four of these animals showed brain pathology, but they had survived earlier studies of poliomyelitis and there was some evidence that the lesions antedated exposure to DDT, and were unrelated to it.

At low dietary levels, other chlorinated hydrocarbon pesticides produce similar histopathology in the rat liver. ORTEGA *et al.* (1957) reported the presence of three stigmata of cellular hepatic disease, including: (1) centrolobular-cell hypertrophy, (2) peripheral margination of basophilic cytoplasmic granulations, and (3) the presence of distinctive cytoplasmic inclusion bodies. These lesions were produced at minimum dietary levels of 2.5 p.p.m. for dieldrin and chlordane and 50 p.p.m. for lindane and toxaphene.

It is generally agreed from studies in a wide variety of species of animals that large doses of DDT can cause liver cell necrosis (HAYES 1959 b), but this effect must not be confused with the reversible effects of small doses discussed above.

It is interesting to note that, in rats poisoned with large doses of chlorinated hydrocarbon pesticides, increases in liver weight and in liver fat content have been noted for DDT (SARETT and JANDORFF 1947) and for dieldrin (DURHAM *et al.*, manuscript in preparation).

DDD has been shown to cause atrophy of the adrenal cortex (NELSON and WOODARD 1949). This finding is of particular interest in view of the fact that, in animals exposed to DDT, high levels of DDT are stored in the adrenal gland in comparison with other tissues.

2. Potentiation. The production of an effect by a combination of two drugs greater than would be expected on the basis of simple additive effect is known as potentiation.

Various combinations of the organic phosphorus compounds have been shown to potentiate each other. Potentiation among these compounds was first shown to occur for malathion plus EPN (FRAWLEY *et al.* 1957). The maximum synergistic effect noted (88 to 134 times) has been observed with malathion plus triorthocresyl phosphate (TOCP) (MURPHY *et al.* 1959). The mechanism by which potentiation occurs has been elucidated and appears to involve the interference of one compound with the metabolism of the second. Thus, EPN apparently interferes with the degradation of malathion (COOK *et al.* 1958, SEUME and O'BRIEN 1960 a) or its more toxic metabolite malaoxon (MURPHY and DUBOIS 1957). The potentiation of malathion toxicity by TOCP is very probably attributable to a similar inhibition (MURPHY *et al.* 1959). SEUME and O'BRIEN (1960 a) have pointed out that the enzymatic degradation of malathion takes place at the carboxyester linkage and to a smaller extent at the phosphate ester linkages, also. However, EPN inhibits only the carboxyester hydrolysis. In a subsequent paper, these workers (SEUME and O'BRIEN 1960 b) reported studies of potentiation involving various compounds containing carboxyester or phosphate ester linkages or both. Species studied included the rat and mouse, which have a high carboxyesterase activity, the American cockroach, which has an intermediate amount of this enzyme, and the housefly, which has only a low carboxyesterase activity. Of some 37 different combinations which were studied, results consistent with the "degradation inhibition" theory were obtained in 30. Some of the inconsistencies, particularly two involving Phosdrin, may be explainable on the basis of what has been termed the "opportunity factor". Thus, degrading enzymes are more effective in reducing the toxicity of phosphorothionates than of phosphates, since they have a longer time to act on the former group of compounds than on the latter. This is due to the time required for the sulfur-containing compounds to be oxidized to the toxic oxygen analogue.

MOELLER and RIDER (1962) gave repeated oral doses of EPN and malathion in combination to volunteers and concluded that no potentiation occurred under these conditions.

It would appear to be quite possible that potentiation could be a factor in the health hazard to workers with severe occupational exposure to mixtures of compounds which are synergistic.

3. Neurotoxicity. The organic phosphorus insecticides are structurally analogous to triorthocresyl phosphate (TOCP), the etiological agent in the numerous cases of so-called "ginger paralysis" or "jake-leg"; a syndrome that occurred in the United States during prohibition days as the result of drinking adulterated Jamaica ginger (Smith *et al.* 1930). Ability to inhibit the enzyme cholinesterase is also shared in common between TOCP and the organic phosphorus insecticides.

Smith and Lillie (1931) established that "jake-leg paralysis" was accompanied by a demyelination of the nerve sheath. In addition to man, only chickens and calves among various experimental animals tested developed this paralytic syndrome. Polyneuritis resulting from the poisoning of industrial workers with TOCP has been reported (Hunter *et al.* 1944). In North Africa in 1959, an estimated several thousand people developed typical paralysis following the use of a cooking oil which had been diluted with motor oil containing TOCP (Smith and Spalding 1959).

In 1946, Koelle and Gilman demonstrated muscle weakness of the hindlegs of dogs, eventually leading to almost complete paralysis, following repeated sublethal doses of diisopropyl fluorophosphate (DFP). In a later paper these authors postulated the identity of this syndrome with "ginger paralysis" (Koelle and Gilman 1949). They further suggested that the syndrome of "ginger paralysis" might be a reflection of the effect of prolonged deficiency of cholinesterase at the neuromuscular junction.

In 1951, a group of three chemical plant workers who were engaged in the manufacture of mipafox (Isopestox) developed acute cholinergic symptoms attributed to intoxication with this compound (Bidstrup *et al.* 1953). These symptoms were ameliorated by atropine and the patients recovered from the acute illness, but ten days later two of the three developed weakness in the legs. In one, the weakness progressed to almost complete paralysis of the legs, with partial involvement of the hands and arms. Ten months later both had residual effects. The more severely affected was still unable to walk two years later. The authors were able to reproduce this syndrome in chickens by dosing them with mipafox.

Barnes and Denz (1953) demonstrated demyelination in the peripheral nerves and particularly in the spinal cord of chickens paralyzed by TOCP, DFP, and mipafox. They pointed out the similarity of the histological lesions in hens poisoned by each of the three compounds.

Although chemical structure, various physical properties, and certain pharmacological effects including particularly pseudocholinesterase inhibition have been studied, no definite correlation has yet been made between any of these properties and ability to produce delayed paralytic effect. Davies *et al.* (1960) have postulated an intermediate role for cholinesterase, whereby a reversible enzyme-inhibitor complex provides the mechanism by which the "toxophore" group (fluoride from DFP or a cresolic derivative from TOCP) is carried to a sensitive tissue site and then released. However, Cavanagh and Holland (1961) surveyed the distribution of cholinesterases in the spinal cord and brain stem of the chicken, rat, rabbit, cat, and guinea-pig and found no simple relation between sites selectively damaged by the neurotoxic organo-phosphorus compounds and centers of cholinesterase activity and no explanation for the variation in species susceptibility. Thus, the work of CAVANAGH and HOLLAND (1961) does not offer support to the hypothesis of DAVIES *et al.* (1960). In a recent paper CAVANAGH *et al.* (1961) have reported that tri-*p*-ethylphenyl phosphate produces neurotoxic effects but is not an inhibitor of cholinesterase.

In view of the serious and essentially irreversible nature of this paralytic syndrome and the widespread usage of various organic phosphorus compounds as insecticides, the suggestion has been made that all compounds with the organic phosphorus grouping should be screened for possible paralytic effect before being admitted to general usage as pesticides (DURHAM *et al.* 1956 b). This concept has now been expanded to include all pesticides having anticholinesterase activity. Thus, the carbamate insecticides are included in the screening program along with the organic phosphorus compounds. The paralytic effect in the chicken appears to be the best available index of a possible paralytic effect in man and is, to date, the screening method of choice for this purpose.

A series of seven compounds, including the more widely used of the organic phosphorus pesticides which had not previously been tested in this regard, were screened for possible paralytic effect by DURHAM and his co-workers (1956 b) using atropinized hens as the test animal. Acute cholinergic effects were seen at higher dosage levels with all compounds tested. No delayed effects were seen following dosage with chlorthion, DDVP, demeton (Systox), diazinon, and schradan. A syndrome different from "ginger paralysis" appeared in the hens dosed with malathion and with EPN.

These chickens developed leg weakness immediately after dosing, in addition to the more usual cholinergic symptoms. The occurrence of general muscle weakness and incoordination as a nonspecific sign in actuely ill, poisoned animals is not unusual. However, the muscle weakness occurring in chickens dosed with malathion and EPN was localized and persisted after the other acute symptoms had vanished. While present, this weakness was clinically indistinguishable from "ginger paralysis". FRAWLEY *et al.* (1956) showed that myelin degeneration occurred in EPN-dosed chickens which developed muscle weakness, but no nerve damage was seen in malathion-dosed chickens which were paralyzed. The paralytic effect of large subcutaneous doses of malathion (minimum effective dose, 100 mg./kg.) and EPN (minimum effective dose, 40 mg./kg.) is not interpreted as an indication that a similar effect in man will result from exposure to these insecticides by the more usual routes, or at expected dosage levels.

4. Carcinogenic effect. The toxic effect of chemicals that probably causes the greatest public apprehension is the tendency to produce cancer.

The problems involved in estimating the carcinogenic hazard of pesticide residues are complex. Some aspects of this problem have been considered in an earlier review in this book series (OSER 1962). Since many chemical compounds are weakly carcinogenic, or require a long period of time to produce their carcinogenic effect, or both, oncological studies require large numbers of animals and long periods of time for completion. Most materials that have been shown to produce cancer in man have been found to produce cancer in several species of experimental animals. However, in certain instances, it has not yet been possible to induce cancer in animals by experimental exposure simulating conditions epidemiologically associated with cancer in man (*National Research Council* 1960). The variability between carcinogenic effect in man and that in experimental animals has been discussed by SALTER (1948). On the other hand, a rather large number and variety of substances are known that induce cancer in experimental animals but have no recognized relationship to cancer in man. There are also differences in susceptibility to carcinogenic effect between some species of experimental animals.

It has been pointed out (*National Research Council* 1960) that one important problem in calculating the carcinogenic effect of pesticides and other food additives which are present in the general diet is the difficulty of making meaningful epidemiological studies. The virtual impossibility of distinguishing those persons in the general population who have not been exposed to pesticide residues in their diet makes comparison of the incidence of cancer for exposed and unexposed groups an impractical approach to the problem. It is usually possible, however, to locate groups of people who have an unusually large exposure to the toxicant in their occupation. Thus, an epidemiologic comparison of tumor incidence can be made between groups with various degrees of exposure to the poison.

It is also possible to compare data on the annual incidence of cancer in certain population groups since the introduction of the modern pesticides with similar statistics for years before these chemicals were used. FOWLER (1953) has made such a comparison for residents of an intensively-farmed area of the Mississippi Delta in the United States for cancer as well as for certain other selected diseases and for deaths from all causes. FOWLER noted that, in the four years following the introduction of modern insecticides into this area, the Delta counties showed a decrease in mortality from tuberculosis, dysentery, pneumonia, and deaths from all causes. Only in heart disease was there a pronounced rise for the Delta and this increase was similar to that for the State as a whole, including the portions where pesticides were used to a much smaller degree. The Delta showed no change in cancer incidence while the State as a whole registered an increase.

Aplastic anemia, thrombocytopenia, agranulocytosis, and various anemias that are not neoplastic may be discussed in connection with the malignant blood dyscrasias because some cases are difficult to distinguish clinically from early cases of leukemia. In 1955, the Council on Drugs of the American Medical Association established a Study Group on Blood Dyscrasias to receive and study reports of suspected cases of purpura, agranulocytosis, and aplastic anemia caused by drugs and other chemicals. A total of 411 drugs have been mentioned as possible etiological agents in the 1,195 cases of blood dyscrasias reported to this Study Group during the seven years of its existence (ERSLEV and WINTROBE 1962). Insecticides have been implicated in some 40 cases. The Study Group has prepared a list of

54 drugs believed to be potentially toxic to the blood cells. This list includes the insecticides chlordane and lindane. The evidence implicating lindane as a possible etiological agent in blood dyscrasias seems to be stronger than that against chlordane. Published reports of blood dyscrasias occurring in association with exposure to lindane include the following: agranulocytosis (FRIBERG and MARTENSSON 1953, MARCHAND *et al.* 1956), pancytopenia (*AMA Committee on Pesticides* 1953), aplastic anemia (STOREN 1955) and leukemia (JEDLICKA *et al.* 1958).

In a review of leukemogenic agents, CRONKITE (1961) has contrasted the meager evidence for chemical leucogenesis with the much more definite indication for the leukemogenic effect of ionizing radiation. He feels that benzene is the only chemical which has been definitely implicated as a leukemogen in man. BOGGS, WINTROBE, and CARTWRIGHT (1962) reached essentially the same conclusion as to etiology in their recent review on the acute leukemias.

HAYES (1961) has studied the statistics for the United States for certain blood dyscrasias in relation to the era of the modern synthetic pesticides. The available statistics indicate no increase in agranulocytosis, aplastic anemia, or purpura. There has been some increase in the incidence of Hodgkin's disease and a striking increase in leukemia and aleukemia. However there was no significant change in the rate of this increase between 1930 and 1959 and, most important, there has been no augmentation of this increase associated with the introduction of DDT or with the more recent introduction of other synthetic pesticides.

Several pesticides have been associated with induction of cancer in experimental animals or in man. For some of the pesticides, there is excellent evidence that the chemicals do have a carcinogenic effect. For other pesticides, the available evidence does not seem to support the conclusion that exposure to the chemical is a cause of cancer. The pesticides which have been so accused are discussed in detail individually below:

(a) Arsenic. The earliest implication of arsenic as an etiological agent in cancer is apparently the work of PARIS (1822) who blamed the arsenic content of fumes from copper smelting works for certain cancerous conditions of man and animals. Suspicion of carcinogenic effect has been limited to the inorganic forms of arsenic. The organic arsenicals have not been implicated. Although PARIS' historic implication against arsenic has been regularly quoted by many later authors, BUTLIN (1892) and KENNAWAY (1925 and 1942) have strongly refuted this early work. FROST (1961) feels that the syndrome which PARIS described was actually a manifestation of selenium toxicity. Massive arsenite therapy, principally used in the form of Fowler's Solution (potassium arsenite, 20 percent in water) has caused keratosis. These arsenic-induced keratotic lesions have occasionally undergone malignant degeneration into epitheliomas. WILE (1912) questioned this etiological relationship. The rarity of this condition is shown by the fact that only 143 cases of "medicinal arsenic" epithelioma could be found in the world literature (NEUBAUER 1947) even though millions of people were treated with arsenicals between 1905 and 1947.

In a very extensive study carried out in the Wenatchee, Washington, area between 1937 and 1940 by NEAL and his co-workers (1941), the health status was determined for 1,231 persons who lived and worked in this apple-growing region, where large quantities of lead arsenate had been used since about 1900. Only seven persons had a combination of clinical symptoms, primarily gastrointestinal in nature, and laboratory findings which suggested acute lead arsenate intoxication. No increase in the incidence of any other disease, including cancer, was noted. In the total group of 1,132 adults, only 12 neoplastic lesions were observed. The authors concluded that, considering the ages of the individuals and the types and locations of the lesions, it did not seem that lead arsenate was a causative factor in the tumors which were found.

The occurrence of cancers of the liver and skin has been reported from Germany for vineyard-workers who were occupationally exposed to arsenical pesticides and for persons who drank wine containing heavy arsenic residue (BRAUN 1958, HESS 1956, KOELSCH 1958, ROTH 1957 a, 1957 b, and 1958). These reports seem to be deficient in that they merely cite the occurrence of cancer in a certain number of vineyard-workers or wine-drinkers, but do not report the size of the total population at risk and do not determine on a statistical basis whether or not the incidence is significantly increased.

All the evidence relating to a carcinogenic effect for arsenic is based on epidemiologic and other studies in man. No experimental data have yet been published showing that any form of arsenic can give rise to tumors in other animals (*WHO Expert Committee on Food Additives* 1961). HUEPER and PAYNE (1962) have recently reported the results of a study in which they attempted to reproduce, in experimental animals, the German observation of carcinogenic effect from lead arsenate. They fed rats for 24 months on high levels (up to 0.8 mg./rat/day) of arsenious oxide in water or in alcohol. No significant increase in the incidence of tumors was associated with consumption of either alcoholic or aqueous arsenic as compared with control rats.

Perhaps it would not be out-of-place in this review of pesticide residues in food to mention the use of arsenical pesticides in the tobacco industry. It has been noted in the United States (HOLLAND *et al.* 1958) that the increase in lung cancer rate has been paralleled by an increase in the arsenic content of the locally-grown tobacco. However, it has been pointed out that, even for heavy smokers, the amount of arsenic contained in tobacco smoke is quite small (DOLL 1955). DOLL also noted that, although Turkish tobacco is almost devoid of arsenic, lung cancer is high among the Turks in Istanbul. The present view of workers in this field seems to be that arsenic does not explain the high incidence of lung cancer in cigarette smokers (FROST 1961).

One of the major problems arising from any consideration of arsenic residues is the fact that this element is a common, naturally-occurring component of many foods, notably fish and shellfish (*WHO Expert Committee on Food Additives* 1961). Milk contains about 0.3 to 0.5 p.p.m. of arsenic on a dry weight basis (FROST 1961). It will clearly never be possible to completely eliminate arsenic from the human diet.

(b) Aramite. Aramite [2-(*p*-tertiary-butylphenoxy)-isopropyl-2-chloroethyl sulfite] is an effective acaracide. It has a very low order of acute toxicity. Aramite was given a tolerance level of 1 p.p.m. and was used on fruit and other food crops in the United States for a period of about eight years. However, long-term toxicological study showed that this compound was a carcinogen both for the rat and the dog. In 20 rats fed 500 p.p.m. of Aramite in their diet for two years, a single focus of nodular hyperplasia was seen (OSER and OSER 1960). The authors interpreted this finding as an indication that the 500 p.p.m. level of Aramite was a "no effect" level. However, this interpretation of the significance of this lesion has been questioned by others. Additional feeding tests and detailed histological studies (POPPER *et al.* 1960) have been carried out. At 1,580 p.p.m., a significant incidence of pathologic liver changes was observed which, in two out of 21 rats were classified as malignant. At 5,000 p.p.m. the hepatic lesions were diagnosed histopathologically as hepatomas or cholangiomas. In a one-year study using three dogs at each dosage level, 500 and 1,580 p.p.m. produced degenerative changes in the liver, but no tumors were noted (STERNBERG *et al.* 1959). However, in a longer study (46 months up to publication of their paper) in which more dogs (40) were used, STERNBERG and his colleagues (1960) found one or more carcinomas in the biliary tract of 15 of 19 animals that died or were sacrificed during this period on diets containing 500 to 1429 p.p.m. of Aramite. Areas affected included the intrahepatic bile ducts, the gallbladder, the common hepatic and common ducts, and the ampulla of Vater. In addition, six of these dogs also showed neoplastic but non-malignant nodules in the liver. These authors commented on the rarity of metastases (only one was observed) and on the contrast between the effect of Aramite on the dog and on the rat. They advanced the possibility that the restriction of carcinogenic effect to the liver in the rat might be due to the absence of the bile-concentrating system in this species.

Following elucidation of its carcinogenic effect in these two species, a zero tolerance was established for Aramite.

(c) Aminotriazole. Aminotriazole is an effective weed killer. It was first registered for use as an economic poison by the U. S. Department of Agriculture in 1957. Approved uses include post-harvest application to cranberry bogs not later than 7 to 10 days after picking, but at no other time; to apple and pear orchards before fruit forms or after picking; and to corn, forage crops, and grain post-harvest or pre-planting. No tolerance level greater than zero has been permitted for residues of aminotriazole in any foodstuff.

In toxicological studies on aminotriazole, the compound was found to have an acute toxicity of a very low order of magnitude. However, it was found that, following a two-week feeding period, rats given 60 or 200 p.p.m. of the compound in their diet showed an enlarged thyroid gland which was associated with a decreased uptake of radioactive iodine (JUKES and SHAFFER 1960). These changes were not seen in rats fed at 15 or 30 p.p.m. (EGGERT 1960). After 24 months of exposure, thyroid anemonas were found in one of ten rats fed 10 p.p.m., two of 15 rats at 50 p.p.m., and 17 of 26 rats at 100 p.p.m. The thyroid glands from one rat fed 50 p.p.m. and from four rats fed 100 p.p.m. showed changes interpreted by some pathologists to be adenocarcinomatous and by others to be non-malignant. The reversibility of these changes is noteworthy. After 17 weeks of feeding at 500 p.p.m., aminotriazole was withdrawn from the diet of a group of rats and two weeks later their thyroids were found to be normal in appearance (EGGERT 1960).

The significance of this thyroid pathology has been discussed by JUKES and SHAFFER (1960). These authors have pointed out that a number of chemicals have the property of inhibiting the thyroid gland from reacting with inorganic iodide to form the thyroid hormone. Many foods, including cabbage, turnips, peas, beans, strawberries, and milk have been shown to contain substances with this type of antithyroid action (GREER and ASTWOOD 1948). In fact, rutabagas have been shown to contain up to 200 p.p.m. of goitrin (*L*-5-vinyl-2-thiooxazolidone) which has a potency in human subjects equal to that of thiouracil (ASTWOOD *et al.* 1949).

Suppression of thyroid function by administration of antithyroid compounds is known to produce an increased supply of the thyroid-stimulating hormone of the pituitary and a consequent increase in the size of the thyroid (GRIESBACH 1941). Evidence from experiments with rats indicates that the antithyroid action of aminotriazole is identical with that of thiouracil and its derivatives (ALEXANDER 1959 a and 1959 b, JUKES and SHAFFER 1960). These effects are similar to those produced by deprivation of dietary iodine. Either the prolonged administration of antithyroid compound or continued deprivation of dietary iodine may lead to the production of thyroid adenomata (GRIESBACH 1941, GRIESBACH *et al.* 1945, AXELRAD and LEBLOND 1953 and 1954). Long continued feeding of comparatively high levels of antithyroid substances, such as thiourea and thiouracil, has produced nodules of thyroid-like tissue in the lungs of mice (MORRIS and GREEN 1951). However, true metastases have not been observed. This lack of a metastatic effect has been regarded as evidence against any direct carcinogenic effect of thiourea (PURVES and GRIESBACH 1947).

The mechanism of the antithyroid effect of aminotriazole seems to be related to an obstruction of iodine uptake by the thyroid (ALEXANDER 1959 a and 1959 b). Aminotriazole inhibits activity of the enzymes catalase and peroxidase in thyroid and in other tissues. Alexander feels that inhibition of thyroid peroxidase is the essential factor in the pathology produced by aminotriazole.

JUKES and SHAFFER (1960) have emphasized the fact that adenocarcinomas have been reported in experimental animals only when antithyroid dosage producing essentially complete inhibition of thyroid activity has been continued for most of the animal's life span.

Thiouracil and other related antithyroid compounds are widely used in clinical medicine in the treatment of hyperthyroidism. There is even some evidence that these antithyroid compounds can, under certain conditions, be useful agents in cancer chemotherapy. Aminotriazole significantly delayed the production of liver cancer in rats that had received 4-dimethylaminoazobenzene, a carcinogenic dye (HOSHINO 1960). Thiouracil has been reported to protect the livers of rats against the carcinogenic action of 2-acetaminofluorene (Paschkis *et al.* 1948). The mechanism of this protective action is not known.

In the United States in 1957 some growers apparently misused aminotriazole, and the U.S. Food and Drug Administration had 3.5 million pounds of cranberries withdrawn from the market on grounds that the berries had not been shown to be safe for human consumption (Du Shane 1959). In 1959 contaminated berries were again found, first in Washington and Oregon and later in Wisconsin. On November 9, 1959 the Secretary of Health, Education, and Welfare issued a public warning that a part of the current cranberry crop was contaminated with aminotriazole and indicated that steps were being taken to remove these contaminated berries from the market. The necessity for this action has been vigorously debated. Clearly, the Food and Drug Administration was responsible under the so-called "Delaney Amendment" to the U.S. Food, Drug, and Cosmetic Act to keep foods containing any residue of a carcinogen off the market.

(d) Other compounds. Among the other pesticides that have been implicated as carcinogens by some workers are DDT, aldrin, dieldrin, and IPC (isopropyl-*N*-phenylcarbamate).

In one of the early studies of DDT in experimental animals, Fitzhugh and Nelson (1947) described the occurrence of a minimal hepato-carcinogenic tendency in rats fed DDT in their diet for two years. Tumors were not noted until after 18 months of dosage. Four rats had one or more small, hepatic cell tumors. These tumors were from 5 to 12 mm. in diameter and paler than the surrounding tissue. Microscopically, they showed an almost complete loss of lobular architecture but were not sharply circumscribed from the rest of the liver tissue. Mitoses were not seen. Some of the cells showed typical DDT pathology of the most severe degree. The authors felt that these tumors could be regarded as adenomas or as low grade hepatic cell carcinomas. An additional 11 rats showed varying amounts of nodular adenomatoid hyperplasia, generally 1 to 3 mm. in diameter. The authors felt that one or at the most two rats from an equivalent control group of this age would spontaneously develop liver tumors in comparison with 15 DDT-fed rats which were affected. This apparent tendency for DDT to produce liver nodules in rats has not been seen in other species studied, including the monkey (Durham *et al.*, 1963), nor have studies in several laboratories using the rat confirmed this early observation. A possible explanation of these dissimilar findings is the fact that technical grade DDT of more recent manufacture contains a higher percentage of the pure *p,p'*-isomer and lesser amount of impurities including the *o,p'*-isomer, the *o,o'*-isomer, and other contaminating materials.

Maier-Bode (1960) found no essential difference in storage of DDT or of DDE between 21 persons who died of cancer and 39 persons who died of other diseases.

Neither benign nor malignant tumors were observed in susceptible mice painted weekly for 52 weeks with five percent DDT in kerosene (Bennison and Mostofi 1950). The treated mice did show acute and chronic inflammatory changes in the painted skin and minimal liver damage. It has even been reported that DDT shows some curative action against cancer (HARTWELL *et al.* 1946), but this action is not considered to be great enough to be of practical use. In this connection, it is interesting to note that the closely related DDD has found use in clinical medicine in chemotherapy of tumors of the adrenal cortex (BERGENSTAL 1960).

DAVIS and FITZHUGH (1962) have studied the tumorigenicity of aldrin and dieldrin for mice. They reported hepatomata in 38 of 215 mice (17.7 percent) fed a diet containing 10 p.p.m. aldrin, in 38 of 218 (17.4 percent) fed 10 p.p.m. dieldrin, and in nine of 217 mice (4.1 percent) fed a control diet. On the average, liver tumors developed in those animals which had been on insecticide dosage for longer periods. The earliest hepatic tumor noted occurred in a mouse which had been on aldrin for 39 weeks.

In calculations of incidence based only on those animals which remained on their respective diets for the full 2-year period, hepatomas occurred in two of two of the dieldrin-fed mice (100 percent), in six of eight aldrin-fed mice (75 percent), and in three of 11 control mice (27 percent). Grossly, the tumors ranged in size from nodules 2 mm. in diameter to 1.5 × 2.5 cm. masses. Microscopically the lesions were hepatic-cell adenomata consisting of expanding nodules of hepatic parenchymal tissue, usually with altered vascular architecture, and morphologically they ranged from very benign lesions to borderline carcinomas. Lung sections were prepared from more than half the mice with hepatomas. No metastases were found. The authors stated that, since the feeding of both aldrin and dieldrin resulted in statistically significant increases in the incidence of hepatic tumors, these compounds are tumorigens. However, since the tumors produced were morphologically benign and since no metastases occurred, aldrin and dieldrin should not be considered carcinogenic.

IPC (isopropyl *N*-phenylcarbamate) and its chloro-analogue (CIPC) are plant-growth regulators used as herbicides and as potato-sprouting inhibitors. Their acute toxicity is quite low. In long-term feeding experiments in mice, rats, and dogs no carcinogenic tendency has been noted (ENGELHORN 1954, LARSON *et al.* 1960). However, when either IPC or CIPC was administered orally to mice for six months and the skin treated concurrently with a solution of croton oil in olive oil, a number of papillomas of the skin were observed (ESCH *et al.* 1958). The difference in incidence of tumors in treated and in control mice was of borderline significance. A number of the treated animals had multiple tumors. Most of the papillomas regressed completely when treatment of the skin with croton oil was stopped, but a few of the tumors developed into malignant squamous-cell carcinomas. There was no evidence that IPC or CIPC influenced the incidence of tumors in other organs (ESCH *et al.* 1958).

Other related carbamate esters, including isopropyl carbamate, have been shown to be carcinogenic (LARSEN 1947).

III. Quantitative dosage-effect relationships for pesticides in man

Information on the amount of any given pesticide which may produce poisoning in man is available from several sources, including use experience data, accidental poisoning cases, and human volunteer studies.

In some instances, it is possible to derive a meaningful figure for the maximum tolerated dosage or for the dosage which produced poisoning. In other circumstances, although use experience with the chemical may be available, or poisoning attributed to it may have occurred, no actual figure is available for the dosage-effect relationship. In certain instances, although no dosage value can be obtained directly, it is possible by indirect means to obtain a figure. For example, in the work of ORTELEE (1958) with DDT formulating-plant workers, although the amount of DDT exposure was not directly known, an estimated dosage figure could be derived from the dichlorodiphenylacetic acid (DDA) excretion levels.

There is also a qualitative difference between data obtained from use experience and that derived from poisoning cases or human volunteer studies. In the latter instances it is usually known that exposure was to a single, specific compound. However, use experience data are generally complicated by the fact that exposure is to more than one compound.

a) Use experience data

In the usual course of manufacturing, formulating, packaging, and applying a pesticide, a number of workmen will receive significant exposure. It has been pointed out that, on the average, poisoning can be expected to appear most quickly, most frequently, most diversely, and most severely in those persons most extensively exposed (*A.M.A. Committee on Pesticides* 1956). For this reason, the study of men with prolonged occupational exposure to pesticides is of particular interest. However, the general population, who are exposed to small quantities of these chemicals as residue in the diet or elsewhere in the environment, represent by far the largest group for study.

In the evaluation of use experience data for all groups, the major problem involves determination of the exposure level. Clearly, demonstration of lack of harm to a certain individual or group is essentially meaningless unless some measure of the degree of exposure involved is available.

The exposure of individuals to insecticide residues on food, as a result of occupational contact, through environmental exposure as a result of drift, or by other means, can be measured for a number of compounds. However, generally the measurement of exposure from residues is more difficult than that from most other sources, particularly from occupational contact, because of the very small quantities of contaminant involved in residues.

1. Chlorinated hydrocarbon compounds. Exposure to the chlorinated hydrocarbon pesticides can be estimated from the storage level of the compounds or of their metabolites in adipose tissue or, in certain instances, from excretion of biotransformation products in urine.

The fact that the chlorinated hydrocarbon materials are stored in adipose tissue in exposed animals is not surprising in view of the known solubility of these compounds in fats and fat solvents and their practical insolubility in aqueous solvents. The compound which has been studied most carefully in this regard is DDT. The storage level of DDT in the fat does not increase indefinitely during continuous exposure but rather reaches a plateau characteristic of any particular level of intake. Not all species show the same concentration of storage when maintained on the same dosage. At any substantial level of intake, female rats store more DDT-derived material than do males, but no difference is apparent at low levels of intake. Partial reversal of these differences in storage has been produced by dosing gonadectomized rats with the opposite sex hormone (Durham *et al.* 1956 a). A sex difference in the storage of DDT has not been demonstrated in species other than the rat.

The earliest published report of DDT storage in man is apparently that of Howell (1948). He found DDT-derived material at a concentration of about seven p.p.m. in the fat of a man with occupational exposure for four years and a history of eating foods known to bear appreciable residues.

Perry and Bodenlos (1950), probably because of technical difficulty, were unable to detect DDT in fats from 16 applicators whom they studied. Only a little later, however, Laug and his co-workers (1951) demonstrated DDT ranging from 0 to 34 p.p.m. (mean 5.3 p.p.m.) in the fat of 75 persons with no special occupational exposure. They found no sex difference in regard to DDT deposition.

Pearce *et al.* (1952) reported that DDE, the dehydrohalogenated metabolite of DDT, was deposited in human fat in somewhat larger quantities than was DDT. Mattson *et al.* (1953) presented additional data on the occurrence of DDE in human fat. Of 50 samples taken from different geographical areas and different occupational groups, 48 (96 percent) showed detectible amounts of DDT and DDE. In no case was DDT found alone. The total DDT plus DDE ranged from 0 to 80 p.p.m., with DDE representing from 39 to 86 percent of the total. There is a negative correlation between the storage level of total DDT-derived material and the percentage of this total represented by DDE. This fact supports a view that individuals with a lower degree of exposure to DDT are able to convert DDT to DDE more efficiently (Durham *et al.* 1961). It would also appear that the time interval during which the DDT has been stored correlates with the degree of its conversion to DDE. Thus, persons whose exposure has been recent show a smaller fraction of the DDT-derived material present in the form of DDE than individuals with earlier or long-continued exposure.

The DDT which is so widely distributed in fat in the general population presumably arises mainly from the consumption of low levels of DDT residue on food. At least a part of the stored DDE probably arises in this way also, since it has been shown that a substantial fraction of the DDT-derived material in the total diet is present in the form of DDE (Walker *et al.* 1954). However, the occurrence of a larger fraction of DDE in total DDT-derived material in human fat (56 percent) as compared to the total diet (33 percent) would seem to indicate that *in vivo* conversion also takes place. Mattson *et al.* (1953) have called attention to the finding of 122 p.p.m. of DDT and 127 p.p.m. of DDE in the fat of an individual who worked in a DDT formulating plant. This high storage level of DDE offers further evidence that conversion from DDT has taken place within the body since it is not likely that this individual was exposed to any significant quantities of DDE in his work.

There are both qualitative and quantitative differences between species with regard to pesticide metabolism. Thus, although monkeys (Durham *et al.*, 1963) and rats (Hayes *et al.* manuscript in preparation) fed DDE store the compound in adipose tissue, and although DDE is found in the fat of rats fed DDT, monkeys on DDT diets store little or no DDE. It appears that the failure of monkeys fed DDT to store DDE is not due to an inability to absorb and to store DDE but rather to an inability to convert DDT to DDE. The monkey does excrete DDA in the urine, as do men and rats. The sex differences for DDT and DDE storage (Durham *et al.* 1956 a) and for susceptibility to parathion (DuBois *et al.* 1955) and to some other organic phosphorus pesticides (Gaines 1960) that occur in rats have not been noted in other species studied. However, not enough is known at the present time about the response in man to either DDT or parathion to know whether or not a sex difference exists.

The most extensive study of DDT storage in man is that of Hayes and his co-workers (1958), who found that the storage of DDT and of DDE in people varied according to their dietary and occupational exposure. Analysis of ten samples of human fat collected and sealed before the advent of DDT in the United States failed to show any trace of DDT or any known metabolite. Abstainers from meat stored, on the average, about half as much DDT and half as much DDE as persons in the general population. The mean daily DDT content of the meatless diet was determined by these authors to be about one-fourth that of the average diet which does contain meat. These findings are in agreement with the report by Walker *et al.* (1954) that foodstuffs containing meat, on the average, had a higher DDT and DDE content than did other food items. The average storage in fat for the general population with ordinary dietary habits, with no occupational contact with DDT, and with little or no environmental exposure to the insecticide was found to be 4.9 p.p.m. for DDT and 6.1 p.p.m. for DDE. Persons in the general population who did have appreciable environmental exposure to pesticides from drift from near-by fields or orchards showed a small but statistically significant increase in storage for both DDT and DDE in comparison with those with no environmental exposure. Occupational exposure resulted in a much more marked increase in the storage of DDT-derived material. Persons who had had moderate, occupational exposure within a year stored DDT at an average concentration of 17.1 p.p.m.

Hayes *et al.* (1956) found from 3 to 13 p.p.m. of DDT (mean 7.4 p.p.m.) and from 5 to 26 p.p.m. of DDE (mean 11.2 p.p.m.) in the fat of volunteers in an institution before the men began exposure.

There apparently has been no progression in the storage of DDT in the general population of the United States between the time this storage was first measured in 1950 (Laug *et al.* 1951) (range 0.0 to 34 p.p.m., mean 5.3 p.p.m.) and the most recent study in 1954 to 1956 (Hayes *et al.* 1958) (range 2 to 12 p.p.m., mean 4.9 p.p.m.). A new survey almost completed in 1962 confirms the earlier finding that there has been no increase of DDT storage in the general population of the United States since storage was first measured in 1950 (Quinby *et al.* manuscript in preparation).

Read and McKinley (1961) reported average levels of 1.6 p.p.m. of DDT and 3.3 p.p.m. of DDE in 62 samples (presumably from 62 different individuals) from different geographical areas in Canada. The content of DDT-derived material ranged from the nondetectible level to 14.3 p.p.m. The authors gave no information on the dietary, environmental, or occupational history of the persons tested, nor did they state in what year the samples were collected.

Maier-Bode (1960) found 0 to 2.66 (mean 1.01) p.p.m. of DDT and 0 to 6.13 (mean 1.17) p.p.m. of DDE in extractible lipid from 60 persons of the general population from different geographical areas in Germany. DDT and DDE levels for both Canadians and Germans are significantly lower than those found for the general population in the United States. An explanation for the apparent difference between DDT and DDE storage levels for the United States and Canada is not available at this time.

Alaskan eskimos store considerably less DDT (range 0 to 1.9 p.p.m., mean 0.8 p.p.m.) and DDE (range 0 to 3.9 p.p.m., mean 2.0 p.p.m.) in their body fat than the general population in the 48 contiguous states. The unusually high percentage of DDE (73 percent for these eskimos versus 56 percent for the general population in the 48 contiguous states) in relation to total DDT-derived material is noteworthy and is thought to reflect the more efficient conversion of DDT to DDE at low storage levels. The virtual absence of DDT from the native diet is discussed below. The small amount of DDT and DDE found in the fat of the native Alaskans is probably accounted for by their limited consumption of imported foods.

DDA, the acetic acid derivative of DDT, is excreted in urine by exposed individuals at a concentration proportional to their fat storage level of DDT and to their absorption of the insecticide. Recent work by Bleiberg and his co-workers (1962) indicates that some of the toxic effects following DDT exposure are explainable on the basis of conversion to DDA. Although DDA is less toxic to experimental animals than is DDT, these authors showed that the metabolite does inhibit choline acetylation, apparently by blocking acetate activation. The DDA effect, however, is probably not significant except in very large exposures to DDT.

Ortelee (1958) conducted clinical examinations and laboratory tests, including determination of DDA excretion, on a group of 40 men with intensive and prolonged occupational exposure to DDT. Estimates of DDT absorption in these men were made by comparison of their urinary DDA concentrations with concentrations obtained in human volunteers on known daily oral dosages of technical DDT. The results indicated that 26 of these men had absorbed DDT at a rate equal to, or higher than, that associated with an oral dose of dissolved DDT at the rate of 35 mg./man/day. The men had been exposed at this rate for 0.5 to 6.5 years (mean 3.5 years) but showed no ill effects as judged by medical examination, their own reports, or their work-attendance records. The author concluded that, with the possible exception of rare hypersensitivity reactions, it was unlikely that chronic DDT poisoning would occur in persons in the general population exposed to DDT as a residue in their diet. This conclusion was based on his finding that ill effects were not found in men who had been exposed to DDT for up to 6.5 years in such a way that they absorbed an average of about 200 times as much DDT as that absorbed by the general population from their diet.

Storage of dieldrin has been demonstrated in the fat of one occupationally-exposed individual (Bell 1960), and excretion of a dieldrin metabolite in the urine of spraymen using this insecticide has been reported (Cueto and Hayes 1961).

2. Organic phosphorus compounds. Exposure to the organic phosphorus compounds generally can be determined by measurement of blood cholinesterase activity. The electrometric procedure of Michel (1949) together with a minor modification of this technique for use with capillary blood samples (Marchand 1952) is probably the most widely used method. There are also available colorimetric (Metcalf 1951), titrimetric (Brown and Bush 1950), and manometric (Ammon 1933) procedures. Witter (1963) has reviewed the methodology in great detail.

Exposure to parathion and other organic phosphorus compounds, which on hydrolysis form *p*-nitrophenol or one of its congeners, can be estimated by determination of these phenolic compounds in urine (Elliott *et al.* 1960). Although applicable to a restricted group of compounds, this test has proved to be a more sensitive measure of absorption than is cholinesterase inhibition (Arterberry *et al.* 1961).

A number of surveys designed to determine the incidence of poisoning from the more toxic organic phosphorus pesticides have been carried out. Some of these studies resulted from complaints arising out of local situations in which insecticides were alleged to be the cause of illness in persons exposed unintentionally and only slightly.

Surveys of this sort have been done in a number of areas having very high usage of pesticides, including Wenatchee, Washington (Sumerford *et al.* 1953, Hayes *et al.* 1957), the Delta area of Mississippi (Fowler 1953, Quinby *et al.* 1958), and Phoenix, Arizona (Ganelin *et al.* manuscript in preparation). In each instance, cases of acute poisoning were found in individuals belonging to groups with heavy occupational exposure to the organic phosphorus pesticides. The degree of illness corresponded, as nearly as could be judged, to the degree of exposure. Cases of mild poisoning resembled severe cases except in degree. Men who had occupational exposure to parathion frequently showed some depression of blood cholinesterase activity, even though they remained well. Illnesses resembling mild poisoning have not been found to be significantly more common in persons living in an agricultural community (including those residing in or adjacent to orchards) if they had no occupational or gross accidental exposure, than to persons living in a nonagricultural area where insecticides were not used. Although no effect on their blood cholinesterase activity level was demonstrated, it has been possible to measure the excretion of *p*-nitrophenol in the urine of people whose only exposure to parathion resulted from their living near treated orchards (Arterberry *et al.* 1961). However, the occurrence of even mild illness was always associated with greater exposure and with the excretion of the parathion metabolite at a much higher rate than that encountered by this environmentally-exposed group. In no instance were insecticides found to be a cause of chronic effects or a contributing cause of asthma, influenza, or any other of a wide range of diseases which some have attributed to DDT or to pesticides generally.

b) Accidental poisoning cases and human volunteer studies

Significant information on the amount of toxicant which produces illness of various degrees of severity is often available from cases of accidental poisoning. In addition, a number of careful studies have been carried out in which human volunteers have been exposed experimentally to measured doses of pesticides. Various dosage routes, including oral, dermal, and respiratory, and single as well as repeated doses of toxicant have been used in these studies. The data most pertinent to the question of health hazards of residues are, of course, derived from the work on oral exposure. Consequently these studies will be reviewed in some detail, while the respiratory and dermal investigations will be summarized more briefly.

1. Chlorinated hydrocarbon compounds. McGee (1956) has studied the incidence of poisoning from the chlorinated hydrocarbon insecticides generally. He cited cases of poisoning from DDT, benzene hexachloride, chlordane, aldrin, and toxaphene. He concluded that these poisonings resulted from accident or suicidal attempt and that the number was remarkably small in view of the widespread use of insecticides and of the carelessness with which they are often handled and stored. Recovery from poisoning, when it occurred, was prompt and not followed by sequelae.

a. Benzene hexachloride (BHC). The fatal poisoning of a five-year-old girl weighing 25 kg. (about 55 pounds) was caused by the accidental ingestion of 4.5 g. of BHC as a 30-percent solution in an unspecified organic solvent (Kwoczek 1950). This represents a dosage of about 180 mg./kg. In a short time she developed difficult breathing, cyanosis, and clonic convulsions. Some of the toxic solution was vomited. Three hours later the child was hospitalized, her stomach was washed out, and she was given therapy for failing circulation. She died within a few hours.

Fatal poisoning has also occurred from the ingestion of lindane, the gamma isomer of BHC. A number of children have been poisoned by eating as little as a part of one (0.33 g.) tablet of lindane intended for use in thermal vaporizers. Joslin *et al.* (1960) have reported a fatal case of poisoning in an 18-month-old child who ingested an estimated one-half of a lindane pellet. A similar case was seen by Storen (1955) in Norway. In this instance, is was estimated that the child swallowed about 6.5 g. of the insecticide.

b. DDT. Hayes (1955 and 1959 b) has reviewed the published cases of poisoning resulting from accidental ingestion of DDT. None of these cases involved residue on food products. In each instance the victim ingested a significant quantity of a rather concentrated DDT formulation or of the technical material. In many cases, it was difficult to differentiate between the toxic effect of DDT and that of the solvent or other constituents of the formulation. The acute oral dosage of DDT necessary to produce illness in man is rather accurately known. Illness in some, but not all, subjects has been produced by ingestion of 10 mg./kg. Smaller dosages generally do not produce illness, although a dosage of 6 mg./kg. produced perspiration, headache, and nausea in a man who was already sickly and who was hungry at the time of eating the compound (Hsieh 1954). Those who have shown illness following ingestion of 10 mg./kg. have not had convulsions, but convulsions have frequently occurred when the dosage level was 16 mg./kg. or higher. Dosages at least as high as 285 mg./kg. have been taken without fatal result. However, vomiting usually occurs promptly after ingestion so that the amount actually retained cannot be accurately determined.

Hayes, Durham, and Cueto (1956) fed volunteers DDT dissolved in corn oil at doses of 0, 3.5, and 35 mg./man/day. Because all the men were on an ordinary diet, these dosage levels were chosen as being about 1, 20, and 200 times, respectively, the ordinary dietary level of DDT [0.184 mg./day (Walker *et al.* 1954)] as discussed more fully below. These amounts are approximately equivalent to 0, 0.05, and 0.5 mg./kg./day, respectively. The highest dosage level chosen was about one-fifth the dosage (2.5 mg./kg./day) estimated to cause transient, mild sickness based on animal experiments and on the smallest single, acute dose of DDT known to produce mild illness in man. Along with four controls, ten men ate the DDT daily for a year and a few of them for somewhat longer. No clinical effect associated with dosage was detected, either by the men themselves or by careful, physical examination and laboratory testing. The storage of DDT in body fat was found to be proportional to dosage. About one year was required for the men to reach equilibrium of DDT storage. A statistically-significant difference for DDT storage was found between groups fed recrystallized DDT and those fed the technical grade material. The physiological basis of this difference is not understood. The concentration of DDT and DDE stored in the fat of the volunteers at apparent equilibrium after the highest dosage was almost as high as that for one man with extensive occupational exposure who showed 648 p.p.m. of DDT and 434 p.p.m. of DDE. Men who ate DDT showed an absolute increase in the storage of DDE.

A method for determining the concentration of DDA in urine was perfected during the course of the study (Cueto *et al.* 1956). *The excretion* of DDA was found to be proportional to the dosage of DDT. It was possible to account for about 20 percent of the DDT administered in terms of DDA excreted in the urine. The authors concluded that a large safety factor is associated with DDT as it occurs in the general diet.

The DDT human volunteer study is being repeated with 21 months of dosage and 27 months of further observation, with emphasis on measurement of DDA excretion and on the loss of DDT from storage following cessation of dosage (Hayes *et al.* manuscript in preparation). In this longer experiment, also, no volunteer complained of any symptom or showed, by the tests used, any sign of illness that did not have an easily recognized cause clearly unrelated to exposure to DDT. The loss of DDT from storage is quite slow. Two years after cessation of dosage, average storage levels have decreased to about one-third of the values attained during the DDT dosage period.

c. Dieldrin. Dieldrin poisonings that have occurred in spraymen applying the insecticide for the control of insect vectors of disease have been reviewed by HAYES (1957 and 1959). In certain programs, 10 to 20 percent of spraymen applying dieldrin have been poisoned. Fatal poisoning from ingestion of dieldrin has been reported (*A.M.A. Committee on Toxicology* 1960).

d. Toxaphene. At least five human deaths have been reported as due to toxaphene. All five cases were children. In three instances the poison was swallowed and it may have been swallowed in the other cases also. In no case could the dosage be determined. It appears that 10 mg./kg. or less leads to nonfatal convulsions in some persons but to no disease at all in other persons.

2. Organic phosphorus compounds. a. Delnav. WEIR and KELLER (1962) gave increasing daily oral doses of Delnav to two male and two female subjects over a 73-day period. A dosage level of 0.15 mg./kg./day (about 10 mg./man/day) produced borderline inhibition of plasma cholinesterase but no effect on erythrocyte enzyme activity. Further study which involved feeding 0.075 mg./kg./day (about 5 mg./man/day) to a larger number of subjects (five men and five women) produced no effect.

b. Demeton. UPHOLT *et al.* (1954) studied the health hazards of eating demeton-treated fruit under controlled conditions. Fourteen volunteers ate one peach each day for 14 days and then ate two apples each day for an additional two-week period. Ten of the volunteers ate demeton-treated fruit and four people served as controls and were given unsprayed peaches and apples. The experimental fruit was sprayed with demeton at the maximum recommended level (one pint of 21 percent demeton per hundred gallons of water) and at the minimum recommended time before harvest (21 days). In addition, eight of the same volunteers ate from three to ten peaches each in a single day. Four days later, three participants each ate 12 or 13 peaches. Two participants ate eight apples each at a single sitting. None of the volunteers showed significant changes in either erythrocyte or plasma cholinesterase activity as a result of eating demeton-treated fruit. The only complaints were occasional mild diarrhea or constipation, which occurred in both control and treated groups. The demeton-treated fruit, especially the peaches, was noted to have an off-flavor which increased with storage (UPHOLT, personal communication). The authors concluded that normally healthy adults could, without any harmful effect, consume each day one apple or peach which had been treated with demeton according to recommendations.

c. Dimefox. Daily oral doses of dimefox given to volunteers at rates of up to 0.0020 mg./kg./day (about 0.13 mg./man/day) for 70 days caused no physiological disturbance and no significant depression of blood cholinesterase (EDSON 1956). Daily doses at the rate of 0.0034 mg./kg./day (about 0.26 mg./man/day) for 70 days caused a slight but definite depression of cholinesterase without any symptomatology.

d. EPN. **Moeller** and **Rider** (1962) reported that five male subjects ingested 6 mg. of EPN in corn oil solution daily for 47 days without any significant effect on plasma or red-blood-cell cholinesterase activity and without detectible adverse clinical effect. However, depression of plasma enzyme activity became evident within a few days after dosage at 9 mg. was begun. Decrease of erythrocyte cholinesterase level did not occur until late in the dosage period (after about 40 to 45 days of dosage) and reached its lowest point approximately three weeks after cessation of dosage. A combination of EPN and malathion appeared to be additive rather than potentiative.

e. Malathion. **Mattson** and **Sedlak** (1960) reported two tests in which a volunteer ingested 11 mg. (0.16 mg./kg.) and 58 mg. (0.84 mg./kg.) of technical grade malathion. The man had no symptoms of intoxication and showed no cholinesterase inhibition. Ether-extractible phosphate in the urine increased quickly after dosage and then decreased to the predosage level within 6 to 16 hours. A total of 19 to 23 percent of the total dose was recovered in the urine in the two tests. The toxicity of aerosols containing malathion was investigated by **Golz** (1959). Groups of four men each received 84 one-hour exposures in 42 consecutive days at dosages of 0, 0.15, 0.6, and 2.4 g. of malathion per 1,000 cubic feet of air. At no time did the men experience any cholinergic symptoms or signs, nor was there any significant or dose-related change in blood cholinesterase. The highest dosage, which was far greater than anything that would result from practical use, was moderately irritating to the nose and conjunctiva.

Hayes and his colleagues (1960) tested the safety of malathion dusting powder for louse control by dusting the bodies and clothing of 39 men five times a week for 8 to 16 weeks with talcum powder containing 0, 1, 5, and 10 percent malathion. Complaints about odor and skin irritation were roughly proportional to dosage. No change in blood cholinesterase was found except perhaps with the ten percent powder. Urinary excretion of malathion-derived material was proportional to dosage. No other changes attributable to malathion were noted and the compound was considered safe for the proposed usage.

Five men given daily oral doses of 16 mg. of malathion in corn oil for 47 days showed no significant effect on plasma or erythrocyte cholinesterase activity and no detectible adverse clinical effect (**Moeller** and **Rider** 1962). When the daily dosage of malathion was increased to 24 mg., depression of plasma enzyme activity became evident within a few days. However, decrease of erythrocyte cholinesterase level did not occur until late in the dosage period (after about 40 to 45 days of dosage) and reached its lowest point approximately three weeks after cessation of dosage. A combination of malathion and EPN appeared to be additive rather than potentiative.

f. Parathion. A single oral dose of 120 mg. of parathion was fatal to a scientist who was investigating the toxicity of the insecticide in the early stages of its development (**Goldblatt** 1950). A single oral dose of 25 to 50 mg. was fatal to a child (**Seifert** 1954). Calculations made from data obtained in an outbreak of parathion poisoning due to contaminated barley

(KANAGARATNAM *et al.* 1960) indicated that the lethal dose of parathion was about 0.1 mg./kg. (see section VI b).

EDSON (1957) studied the effect of various dosage levels of parathion given orally to volunteers for five days each week. Both male and female subjects were used in the test. The parathion was prepared as a stock solution in ethyl alcohol and diluted with water shortly before ingestion. Groups of 4 volunteers each were given doses of 0.6 mg. for four weeks, 1.2 mg. for ten weeks, or 2.4 mg. for ten weeks. After four weeks dosage at the lower level, the group on 0.6 mg. per day was switched to 4.8 mg. per day and continued on this higher dosage for an additional nine weeks. Another group ingested daily doses of 7.2 mg. for six weeks. No illnesses occurred in any of these subjects. No detectible effect on whole blood cholinesterase activity occurred following ingestion for ten weeks of dosages of 2.4 mg. per day or less. A questionable cholinesterase effect (92 to 97 percent of the control level) was noted after eight to ten weeks at 4.8 mg. The group fed 7.2 mg. of parathion per day showed a progressive decrease in whole-blood cholinesterase activity, reaching an average minimum level of 67 percent of the control value after six weeks of dosage. The authors concluded that the highest ineffective dosage for parathion in man was about 0.05 mg./kg./day, corresponding to about 3.5 mg./person/day. This amount is equivalent to the consumption of about 3 kg. of foodstuff containing 1 p.p.m. of parathion.

RIDER *et al.* (1958) studied the effect of daily oral doses of parathion in corn oil on human subjects. The ingestion of up to 0.050 mg./kg./day or about 3.5 mg./man/day (the highest level tested) for three weeks following nine weeks on lower dosages produced no significant lowering of either plasma or erythrocyte cholinesterase level. A significant increase in the mean plasma cholinesterase level without an accompanying change in the red-cell enzyme occurred early in the study at dosage levels between 0.003 and 0.025 mg./kg. A slight rebound in the mean plasma cholinesterase was noted immediately after completion of the study. No adverse clinical effects were noted in any of these subjects. The results of this work correlate well with the results reported above by EDSON (1957). However, RIDER and his colleagues did not give dosages as high as those at which EDSON observed depletion of cholinesterase.

g. Schradan. Twelve subjects (six males and six females) ingested 1.4 mg. of schradan in water five days per week for 44 days (EDSON 1955). This dosage was chosen as the equivalent of a daily dose of 1 mg., the amount of schradan in 12 oz. (340 g.) of a foodstuff containing 3 p.p.m. of the residue. No adverse clinical effects were noted, but a definite, slow decrease in blood cholinesterase activity occurred. This decrease was more evident in the erythrocytes than in the plasma. The enzyme depression began within the first two weeks of schradan ingestion. After 18 days of dosage, whole-blood cholinesterase activity for the treated group had fallen to about 80 percent of the control level, but during the following 26 days' dosage, only about five percent further decrease occurred. The authors interpreted their data as suggesting that a state of equilibrium with regard to cholinesterase activity was being attained after about six weeks' dosage at this level despite continuing intake of schradan. One subject ate 4.2 mg. of schradan per day for 74 days. This dosage level caused a marked depression of cholinesterase activity, which reached its lowest point — 23 percent of normal activity in red cells and 50 percent in plasma — after about two months' dosage. On two occasions this subject took single doses of 12.6 mg. in order to compensate for accidental omission on the two previous days. No signs or symptoms of poisoning were associated with consumption of the pesticide. The subject had an entirely normal capacity for exercise and work even at the time of maximum cholinesterase depression.

h. TEPP. Fatalities from TEPP have apparently all involved suicide or gross carelessness such as taking the compound by mouth or spilling a concentrate of it on the skin. The dosages in such cases are, of course, unknown.

Some information on the toxicity of TEPP to man has accrued from the use of this compound in the treatment of myasthenia gravis (GROB and HARVEY 1949). A single parenteral dose of 5 mg., or 3.6 mg. daily for two days, or 2.4 mg. daily for three days, or of 7.2 mg. orally every three hours for three to five doses, produced symptoms in normal subjects, as did slightly larger doses in myasthenic patients receiving atropine. Symptoms at these relatively low dosages included localized fasciculations, anorexia, nausea, sweating, abdominal cramps, salivation, giddiness, restlessness, insomnia, paresthesias, and dreaming. Symptoms appeared suddenly about 30 minutes after the final dose. GROB and HARVEY (1949) have given evidence that 5 mg. intramuscularly or 25 mg. orally would cause severe symptoms. Likewise, they believe that 20 mg. intramuscularly or 100 mg. orally would cause death.

3. Other insecticides. Dinitro-orthocresol. HARVEY *et al.* (1951) found that two of five men given 75 mg. of DNOC (4,6-dinitro-*o*-cresol) daily for five to seven days developed headache, lassitude, and malaise near the end of the test. The symptoms corresponded with sustained blood levels of 20 p.p.m. of DNOC and with peaks of 40 and 48 p.p.m. All subjects showed distinct yellow coloration of the sclera by the fourth day of the experiment. These authors showed that exercise increased the level of DNOC in the blood. The effects of heat were not investigated in this study. However, clinical experience with other dinitrophenols and epidemiological evidence in spraymen indicate that temperature is an important factor in poisoning and the results of animal experiments confirm this opinion.

IV. Residues in food and water

Analytical methods for determining residues of pesticides in food have been reviewed in some detail by GUNTHER and BLINN (1955), SCHECHTER and HORNSTEIN (1957), WESTLAKE (1959), and DURHAM (1961).

The most promising recent development in the analytical chemistry of pesticide residues has been the introduction of gas chromatography in its various forms (SCHECHTER and WESTLAKE 1962). Gas chromatographic purification and separation coupled with determination by thermal conductivity, flame ionization, coulometry, or electron-capture methods have been successfully applied to the determination of chlorinated hydrocarbon and organic thiophosphorus compounds. Although many currently-used methods will detect residues of the order of fractions of a part per million, some of the newer techniques, such as electron-capture gas chromatography or neutron activation analysis, have sensitivities in the parts per billion range (SCHECHTER and WESTLAKE 1962).

In view of the great sensitivity of some of these newer methods, it is of interest to re-examine the concept of zero tolerance. U. S. Food and Drug Administration regulations at present require a zero tolerance for residues of any pesticide known to be a carcinogen and permit no tolerance higher than zero for residues of any pesticide in milk. SCHECHTER and WESTLAKE (1962) have noted some of the problems involved in the zero tolerance concept. The more capable the chemist and the better his techniques and apparatus, the greater will be his chances of detecting very small amounts of residual pesticides. At best, the chemist can only say that, using a specified method and based on analysis of a certain sized sample, the contaminant under study is not present at levels equal to or above the limit of sensitivity of the method. MULFORD (1961) has discussed the implications of the zero tolerance clause and pointed out that an improvement in an analytical method to detect minute amounts of a substance may render food illegal today that was legal yesterday.

There would seem to be valid reason for giving special consideration to the assignment of a tolerance for pesticide residues in milk. However, scientists who have studied the matter are far from agreement on the desirability of a zero tolerance. DECKER (1959) has pointed out that the problems associated with the appearance of pesticides in milk tend to be more psychological than biological in nature.

Other commentators have pointed out the occurrence of DDT in human milk (LAUG *et al.* 1951) and commented that it is inconsistent to require a lower DDT content in cow's milk than occurs in mother's milk. It seems that milk producers, as long as they are able to comply with the zero tolerance, even though with difficulty, are not displeased with the regulation since it fits in with their general advertising of milk as "nature's most perfect food".

The significance of pesticide residues in specific foodstuffs is discussed below.

a) Raw agricultural produce

In 1960, a total of over 2,000 tolerances had been established in the United States involving over 100 pesticidal chemicals (ROE 1960). There have been innumerable studies done and papers published on the subject of residues of the various pesticides on raw agricultural produce. It is beyond the scope of this review to summarize the existent mass of data on residues. Residue data for the chlorinated hydrocarbon materials has recently been reviewed by MARTH (1962).

Plants and plant products may become contaminated with pesticides in at least the following three ways: (1) through direct application of the chemical to the plant in an attempt to control pests, (2) through drift from adjacent spraying or dusting operations, and (3) from soil containing residue from earlier pesticide applications, possibly from previous years.

Information is available from the U.S. Food and Drug Administration and from the State of California that gives an indication of the amount of raw agricultural produce in the United States containing pesticide residues in excess of the tolerance levels.

During 1961, a total of 385 tons (349 metric tons) of raw agricultural commodities containing unpermitted pesticide chemicals or residues in excess of established tolerance levels was seized by the U.S. Food and Drug Administration (*U.S. Department of Health, Education, and Welfare* 1962). Eight of the seizures involved grain. In each instance, the illegal lots of grain had been contaminated by the admixing of seed grain which had been treated with, and bore residues of, mercurial fungicides. The other seizures included leafy vegetables with illegal residues of parathion, toxaphene, and DDT and peanuts with excess DDT. In 1960, there were 60 seizures totalling 1,377 tons (1,249 metric tons). Most notable among these actions were the seizures of cranberries containing residues of aminotriazole and of apple pomace contaminated with DDT. Thirty-six seizures were made in 1958 and 32 in 1957. Fewer seizure actions for over-tolerance produce were necessary in 1961 than in preceding years.

The *California State Department of Agriculture* (1961) tested 2,166 samples of raw agricultural produce for pesticide residues during 1960. Residues in excess of tolerance were found on 141 (6.5 percent) of these samples. In 1959, of the 1,748 samples tested, 154 (8.8 percent) carried more than the allowable quantity of residue. In 1958, 1,846 samples were analyzed and 153 (8.3 percent) were found to be in violation. In their reports, the Department emphasized the fact that the proportion of contaminated samples is higher than would be found by random sampling of all produce offered for sale, since inspectors selectively sample those commodities that for some reason are suspected of carrying excessive residue.

Some writers have expressed concern that the average level of pesticide residue in our general food supply is continually increasing. However, available evidence suggests that no such progression has actually occurred. There was not, as pointed out above, any significant change in the storage of DDT in the general population in the United States between 1950 and 1962. As an indication of change in the residue situation with time for one particular food item, WOLFE *et al.* (1959) reported on the trend between 1948 and 1958 of DDT and parathion residues on apples grown in central Washington. These workers found that residues of DDT and parathion on apples sprayed during the 1958 season in central Washington were only slightly higher than in 1948 and 1954, although there had been an increase in dosage and in number of cover sprays during this period. In almost all instances, the residues were below the tolerance levels established by the Food and Drug Administration.

There are no recorded instances in which application of pesticide according to recommendations has resulted in seizure of the crop for excessive residue. Usually, when an excessive amount of residue is found on a crop, there has been an obvious, gross disregard of recommended practice regarding the amount or timing of the pesticide application, or both. A reasonable safety factor is included in the recommendations for application. This is in addition to the safety factor of 100 or more which is taken into account in setting the tolerance value.

The provision in the pesticide tolerance regulations which the individual grower is most likely to violate relates to mixed residues. Regulations of the *U.S. Food and Drug Administration* (1962) state that, where two or more pesticides with similar pharmacologic actions are present, the combined percentile residues shall not exceed one-hundred. It would thus be possible, at least theoretically, for the farmer to apply each individual pesticide in complete accordance with recommendations, yet have his crop be over-tolerance with regard to mixed residues.

Some studies have been carried out on the problem of mixed residues. During the 1957 season in North Central Washington, pear psylla became refractory to parathion, and the chlorinated hydrocarbon insecticides dieldrin and toxaphene were used by many growers in an attempt to control this insect (DURHAM and WOLFE 1959). These pears were generally treated with DDT also. Among a total of 145 lots of pears analyzed, no residue for a single insecticide was found to be above the tolerance level. However, one of the 45 samples (2.2 percent) sprayed with both DDT and dieldrin and two of the ten samples (20 percent) sprayed with DDT, dieldrin, and toxaphene exceeded the allowable *combined* tolerance.

b) Meat, milk, and eggs

It has been pointed out above that the chlorinated hydrocarbons are lipid-soluble and tend to accumulate in body fat in animals exposed to them. As a consequence of direct treatment with insecticides or of ingestion of insecticide-contaminated forage crops or pasture, fat from meat animals may contain residues of these chemicals. The U.S. Food and Drug Administration has granted tolerance levels for residues of Co-Ral (1 p.p.m.), DDT (7 p.p.m.), lindane (7 p.p.m. or 4 p.p.m., depending on the species), malathion (4 p.p.m.), methoxychlor (3 p.p.m.), and toxaphene (7 p.p.m.) in the fat of meat from various animals, including cattle, goats, hogs, horses, poultry (malathion only), and sheep (*U.S. Food and Drug Administration* 1962).

There have apparently been no surveys of market meat to determine the extent to which contamination with residues of pesticides occurs. However, WALKER and his co-workers (1954), in studies of complete meals which are discussed in more detail below, found that, in general, foodstuffs containing meat, meat combinations, or butter had a relatively high content of DDT. HAYES *et al.* (1958) noted only very small quantities of DDT in "meatless" meals and the storage of only very small concentrations of DDT-derived materials in the adipose tissue of meat abstainers.

Lactating animals that have been exposed also excrete the chlorinated hydrocarbon materials in the lipid moiety in their milk. Since milk is the primary food for infants and invalids who might be unusually susceptible to the effects of toxicants, the U.S. Food and Drug Administration has not granted a tolerance level higher than zero for residue of any pesticide in milk (*U.S. Food and Drug Administration* 1962). Thus, the movement in interstate commerce of milk containing any amount whatsoever of any insecticide is illegal. However, in practice it has not been feasible to take legal action against milk containing very small concentrations of pesticide residue, although surveys have shown that a considerable proportion of the market milk shipments contain small but detectible amounts of pesticide (CLIFFORD 1957). Many states have a regulation similar to the Federal law banning transport or sale of pesticide-contaminated milk within the state. Canada has not yet established a tolerance for any pesticide in either meat or milk.

Surveys have been carried out by the U.S. Food and Drug Administration to determine the extent to which pesticide residues occur in market milk. Small-scale surveys in 1948, 1949, and 1951 indicated that traces of DDT could be found in about 25 percent of market milk samples. In 1955, a detailed survey was carried out, including 50 milk samples from each of the 16 Food and Drug Districts (CLIFFORD 1957). Of the 801 milk samples tested, 496 (62 percent) contained pesticide residues when tested by bioassay. One-hundred and sixty of these samples containing the highest quantities of residual pesticide were subjected to paper chromatography in an attempt to identify the specific compound present. BHC, which was detected in 96 samples (60 percent), was the insecticide encountered most commonly. DDT (86 samples), DDE (57 samples), lindane (42 samples), DDD (39 samples), and methoxychlor (five samples) were also found. Members of the aldrin group (aldrin, dieldrin, and endrin) were not encountered, nor were heptachlor, chlordane, and toxaphene. The authors felt that it was possible that small quantities of the latter two materials might have escaped detection. DDA, a known metabolite of DDT, was not detected. The authors commented on the fact that the repeated finding of DDD was surprising since this compound is not a known metabolite of DDT. The quantitative tests which were done showed that up to 1.5 p.p.m. of DDT or related compounds or both were present. Residues of organic phosphorus pesticides were not detected.

A follow-up survey was carried out from August through September 1958 (CLIFFORD *et al.* 1959). A total of 936 milk samples from the 16 Food and Drug Administration Districts were tested for residues of chlorinated hydrocarbon pesticides, and 23 (2.5 percent) showed residues of 0.1 p.p.m. or more. Using a bioassay comparable to that used in the 1955 survey, 33 percent of samples were found to have detectible contamination. The authors felt that there had been a definite improvement in the milk residue picture between 1955 and 1958. The survey did not reveal any seasonal differences in the occurrence of pesticide residues in milk. An attempt was made to learn the source of the contamination for eight dairies whose milk showed substantial residues. Feeding of DDT-contaminated corn silage (1.2 to 12 p.p.m. of DDT) was found to be responsible for the high residues of DDT (4.8 and 6.3 p.p.m.) in one producer's milk. The contamination in the cases of two other producers was attributed to the careless spraying of the dairy barns with a concentrated DDT solution by a commercial applicator. The source of contamination could not be definitely accounted for in the other five cases.

In a separate study, **CLABORN** *et al.* (1950) investigated the source of milk contamination when dairy barns were sprayed with DDT. Their work indicated that the insecticide excreted in the milk came primarily from residues left on the feed troughs. Contamination did not occur when the feed troughs were completely covered during spraying or were washed afterwards. The insecticide was shown to be actually excreted in the milk rather than being introduced by mishandling the milk after its secretion.

MANN *et al.* (1950) reported that various processing operations including pasteurization had little or no effect on the DDT content of milk fat. They found raw whole milk, raw skim milk, raw cream, pasteurized whole milk, pasteurized cream, butter, buttermilk, whey, and cheddar cheese to have an essentially constant DDT content in their fat moiety.

In connection with the excretion of chlorinated hydrocarbon pesticides in milk, it is interesting to note that **ZWEIG** and his co-workers (1961) have reported that cattle fed a diet containing 0.5 p.p.m. of DDT did not excrete a detectible quantity (0.01 p.p.m. or less) of the pesticide in their milk. At levels of 1, 2, 3, and 5 p.p.m. of added DDT, detectible residues were found in the milk of all animals. DDT concentration in milk was proportional to DDT level in feed. In view of this work, the State of California has established a 0.5 p.p.m. tolerance level for DDT in feed for dairy animals and 7 p.p.m. in feed for other animals (**ROLLINS** 1961). The Federal regulations in the United States permit no residue of DDT on alfalfa hay and other cattle feed moved across state boundaries.

The strict enforcement of the zero tolerance for pesticide residues in milk has essentially eliminated the use of various agricultural by-products as feed for dairy animals. Among the by-products which have been used in the past as cattle feed are apple pomace, corn stover, and pea hulls.

It has been reported that young heifers fed on apple pomace gained weight significantly faster than similar animals fed their usual ration (**PRIODE** and **BURRIS** 1958). However, **DURHAM** and **WOLFE** (1959) found from 6 to 18 (mean 11) p.p.m. of DDT in nine wet pomace samples and from 32 to 46 (mean 36) p.p.m. of DDT in an equal number of dry samples. The *U.S. Food and Drug Administration* (1958) has ruled that apple pomace containing any amount of DDT is unsuitable as a feed for dairy animals, and that care should be taken in feeding apple pomace containing DDT to meat animals to be sure that the meat from them does not contain residues in excess of the 7 p.p.m. tolerance.

As mentioned above, **LAUG** and his co-workers (1951) found detectible quantities of DDT (0.01 to 0.77 p.p.m.) in 30 of 32 samples (94 percent) of human milk tested. The mean concentration in whole milk for all samples tested was 0.13 p.p.m. The DDT concentration in the fat moiety of breast milk, based on these results, is estimated to be about 3.2 p.p.m.

It has been shown that some chlorinated hydrocarbon pesticides, including endrin (**TERRIERE** *et al.* 1959) and DDT (**HAYES** 1959) can be detected in eggs following incorporation into the diet of laying hens. There have apparently been no surveys made of market eggs to determine their content of pesticide residue. However, as discussed below, **Hayes** *et al.* (1958) noted that dishes containing eggs contributed heavily to the DDT content of meatless meals.

c) Complete, prepared meals

The study of residues on individual items of food is of importance as a general protective measure, since this knowledge enables regulatory authorities to take action against those foods which have illegal residues. However, of greater importance for public health is a knowledge of the amount of residue actually consumed by the general population. This knowledge cannot be obtained with sufficient accuracy by calculation or extrapolation. Many food dishes are complex mixtures of many items. It is not valid to assume that items of the diet which are not commonly sprayed are dependably free of pesticide (**Hayes** 1959 b).

The DDT and DDE content of complete, prepared meals from restaurants and institutions were determined by **Walker** and his co-workers (1954). A total of 179 individual portions of food, representing 86 different items, was included in the 18 restaurant and seven institutional meals tested. Fifty food portions contained no detectible DDT; however, DDT was found to be present in detectible but very small quantities in all meals tested. Generally those foodstuffs cooked in fat and those containing meat or butter were found to have a higher DDT content than other foodstuffs. The DDE content of the various foods tested was roughly proportional to the DDT content. If in one day an individual had consumed the three meals that contained the largest amounts of DDT, his total DDT intake would have been 0.388 mg. The average DDT intake based on all meals tested was 0.184 mg./day. This amount is equivalent to a DDT dosage of about 0.0026 mg./kg. for a man of average size (70 kg.) or to a DDT concentration of about 0.31 p.p.m. in the total dry diet. **Walker** and his colleagues (1954) felt that no meals tested contained enough DDT to be considered a toxicological hazard on the basis of the estimated chronic oral toxicity of the compound.

Hayes *et al.* (1956) determined the DDT and DDE content of 16 meals taken from the inmates' dining hall in a penal institution. The mean daily average DDT intake was found to be 0.202 mg. and this value was considered to be in reasonable agreement with the value of 0.184 mg. reported by **Walker** *et al.* (1954), as noted above. **Hayes** *et al.* (1956) found an average daily DDE intake of 0.050 mg., which is only about one-fourth the DDT ingestion for the comparable period.

Of 66 individual items tested from 11 meatless meals, only nine contained detectible DDT and only three contained over six micrograms of DDT per serving (**Hayes** *et al.* 1958). Each of the latter three dishes contained eggs in one form or another. Vegetables showed low DDT residues in the meatless meals just as they did in restaurant and institutional meals, as noted above. The meatless meals contained 36 percent of the DDT-derived material in the form of DDE, a value essentially identical with that for ordinary meals (33 percent). The mean daily DDT content of the meatless diet was determined to be 0.041 mg. This low dietary DDT level is consistent with the low DDT storage level found in the body fat of people who do not eat meat.

Sample meals for one day from a hospital in Alaska were analyzed by DURHAM *et al.* (1961) and found to contain 0.184 mg. of DDT and 0.026 mg. of DDE. Although these daily totals for DDT and DDE were similar to those found in representative restaurant and institutional meals in the 48 contiguous states, the distribution of contamination among the various food items was different. A dish of spaghetti and meatballs contained 0.111 mg. of DDT and represented 60 percent of the daily total residue of this insecticide in the hospital diet on that day. A total of 42 samples of food, representing 31 different items of the Alaskan Eskimos' diet, were also analyzed. Although a limited number of imported food items are consumed, the items tested make up the major portion of the diet of the village eskimos. No DDT or DDE was detected in any of the native foods analyzed, with the exception of the meat from two white owls, each of which contained 1.1 p.p.m. of DDE. Consistent with this low dietary insecticide level, very small amounts of DDT and DDE were found in the body fat of these native Alaskans.

d) Water

The possibility of pesticide residues in potable waters must be considered in evaluation of the hazard to man. Pesticides may enter the water supply by direct, intentional application; by inadvertent drift into water from adjacent spraying operation; or, perhaps, more commonly, by washing from pesticide-treated areas within the watershed.

Fortunately, the great susceptibility of fish to many of these insecticides gives an easy clue to the significant contamination of streams. For example, endrin is toxic to certain species of fish at concentrations of less than 1 p.p.b. (part per billion). The toxicity of toxaphene to fish is of the same order of magnitude as that of endrin. The presence of many of the other chlorinated hydrocarbon pesticides at levels as low as a few parts per billion can be detected by their effect on fish. The carbamate insecticide Sevin and the organic phosphorus compound Guthion also fall within this range of toxicity to fish. The latter group of pesticides are not of particular importance as water pollutants, however, due to their relatively rapid hydrolysis.

Utilizing the high toxicity of the newer synthetic pesticides to fish, the U. S. Public Health Service instituted a nationwide survey of fish kills as a means of detecting water pollution. In the first 4 months of this survey (June through September 1960) more than 200 individual reports of fish kills were received. In 76 cases (38 percent), agricultural chemicals were implicated as the etiological agent (COTTAM 1960).

DEAN (1960) has emphasized the tremendous diluting, entrapping, and reacting influences that pesticides are subjected to in the soil. He points out that an acre-foot of soil weighs some four million pounds and when moist will contain 30,000 gallons of water[6]. This entire mass is available for dilution and entrapment of pesticides applied to the cropland.

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[6] A hectare-meter of soil weighs some 15 million kilograms and when moist will contain about one million liters of water.

Nicholson (1962) has made a very thorough study of the pesticide content of a river system which drains a 400-square-mile cotton-farming area of Alabama in which pesticides, particularly toxaphene, DDT, and lindane, were intensively used. Toxaphene (maximum level 0.0004 p.p.m.) and lindane (maximum level 0.00075 p.p.m.) were detected in the stream. DDT was not detected. The level of pesticides in the water was not decreased by the municipal water-treatment process. Toxaphene was found in the water all year long, but heaviest concentrations coincided with application to the fields. The contamination was general throughout the watershed. The insecticides apparently ran off the land surface dissolved in water. No effects of these pesticides on aquatic life of the stream were noted.

The occurrence of pesticide residue in water has received much less study than has the occurrence of these materials in food. A number of studies of this matter are going forward at the present time. Evidence presently available would indicate that pesticide residues may be present, although at extremely low levels — in the range of less than 0.001 p.p.m. — in streams draining watersheds that have been treated with the chlorinated hydrocarbon pesticides. At a concentration level of 0.001 p.p.m., an individual's average total daily intake of two liters of water would represent only about 0.002 mg. of residual pesticide. According to our present knowledge, this amount of even the most toxic pesticide would be significantly below the level that is hazardous on an acute or subacute basis.

V. Harmful effects from naturally-occurring food constituents

In considering the possible deleterious effects to the human population from small residues of pesticides or other foreign chemicals in food, one should not lose sight of the fact that small quantities of toxic chemicals occur naturally in some foods.

Harris (1962) has pointed out the influence of the various cultures on the diet of civilized man. He has given examples to illustrate the fact that many of the foods in our diet are not necessarily chosen on the basis of their nutritional value and wholesomeness, but that geography and custom exclude a number of otherwise suitable foods from our table-of-fare. Although, in general, foods of animal origin are nutritionally superior to foods of plant origin and although the latter may actually contain substances which interfere with human nutrition, foods of animal origin are more commonly rejected, and with deeper feelings of disgust, than are foods from plant sources.

Probably the best-known example of a toxic natural product in food is the oxalate which occurs in cabbage, spinach, rhubarb, and certain other vegetables. Uncooked soya beans contain an antitrypsin which influences digestibility; cotton seed contains gossypol; and some nuts and manioc contain cyanide precursors. Cereals contain phytate, which has been incriminated as interfering with calcification. Saponins are present in many foods, including potato, soya, beets, spinach, bread, fruit, peanuts, calabash, tomato, and orange (Darby 1960). Astwood *et al.* (1949) isolated from the edible portions of certain vegetables of the mustard family, notably the rutabaga or yellow turnip, *L*-5-vinyl-2-thiooxazolidone, an antithyroid compound that accounted for the goitrogenicity of these foods. Astwood (1960) has estimated that Swedish turnips may be 100 times as potent with regard to antithyroid activity as cranberries "badly contaminated" with aminotriazole. Sitxy-three parts per million of 2-phenyl-isothiocyanate, a compound which was subsequently found to be a potent insecticide, was extracted from turnips. Evidence is accumulating which points to a relationship between ingestion of saturated fat and the occurrence of atherosclerosis (Bronte-Stewart 1961).

Many compounds that occur naturally in foodstuffs are beneficial in small quantities but can be toxic in larger amounts. Vitamins A and D, although essential dietary constituents, can produce serious symptoms of hypervitaminosis A or D with sufficient overdosage. Cobalt, in its role in the formation of the vitamin B_{12} molecule is essential for man (Underwood 1956), yet the continued ingestion of rather low quantities of cobalt produces polycythemia. Grossly excessive intakes of riboflavin (Unna and Greslin 1942) or of folic acid (Clark *et al.* 1953) lead to kidney damage in rats. An excess of lactose in the diet of the young rat produces cataract (Mitchell and Dodge 1935).

An example which comes quite close to home for many of us relates to the fact that an excess of calories from our daily food intake leads to obesity, with its increased risk of hypertension, diabetes, and other disease states. Szepsenwohl (1959) has reported that excess intake of eggs can produce cancer in mice. Mulford (1961) has cited the example of selenium, which as a trace element is a dietary essential, in larger quantities is a toxic substance, and under certain conditions is a carcinogen.

The toxicity of excess sodium chloride has been studied. In rats fed a complete, purified diet to which was added various levels of sodium chloride, animals receiving diets containing salt levels of 2.8 percent had, on the average, higher systolic blood pressure; signs of hypertension including lipemia, hypercholesteremia, and lipid deposits in the renal parenchyma and arterioles; and a shorter life span than control rats (Meneely *et al.* 1957).

The concept that the desired nutritive or therapeutic effect and the toxic or side-effect of various chemicals merely represent different points on a single dosage curve has been discussed in some detail by Riker (1962). In this connection, in a few instances attention has initially been directed to a beneficial nutrient as a result of the illness caused by an excessive intake. Fluorine first came to medical attention when it was learned that residents in areas where drinking water contained more than about 5 p.p.m. of fluorine developed mottled tooth enamel while persons exposed to higher concentrations were subject to bone disease. Now, smaller concentrations of fluorine in drinking water have been found useful in the prevention of dental caries.

VI. Human poisoning cases from residues

a) Cases involving agricultural usage

Two types of injury must be considered in assessing the health hazards of pesticide residues — first, the possibility of an acute illness resulting from ingesting residues on a single day or in a few days; and secondly, the long-term effects that may accrue after ingesting small quantities of residues daily for many years. On the basis of our knowledge of the acute toxicity of the various pesticides to experimental animals and to man, and of the levels of the chemicals present as residues on food products, illness from short-term exposure would not be expected to occur. This conclusion is supported by epidemiologic evidence. Thus, there have been no known cases of illness in the United States from insecticide residues on food when formulations have been used according to directions (HAYES 1960). The same is true in the United Kingdom.

However, there have been several instances in which insecticides used quite improperly on foods have led to acute poisoning soon after ingestion. Nicotine sulfate residues caused illness in 11 persons who ate mustard greens that a farmer had sprayed with double the recommended dosage and then sold the next day (LEMMON 1956). Even two weeks after spraying, the residues on this particular crop were 69 to 123 p.p.m. The tolerance level is 2 p.p.m.

Two families were poisoned when toxaphene sprays were applied contrary to directions to collard greens and chard (MCGEE *et al.* 1952). The residue on the collards averaged 3,315 p.p.m. Even after the greens were washed three times, 3,126 p.p.m. was left. If toxaphene is applied to such crops at all, it is supposed to be used on seedlings only, and in such a way that no residue is present at harvest.

In northern California, during 1959, an individual became ill after eating part of one grapefruit out of a plastic bag containing six grapefruit brought in from another State (*California State Department of Agriculture* 1961). Analysis showed that all the grapefruit were heavily contaminated with cyanide. Surveys made throughout the State and particularly of other shipments made by the grower of the contaminated lot did not reveal any additional grapefruit with cyanide residue. Intensive investigation failed to yield any explanation. Cyanide fumigation of citrus fruits and other commodities brought into California has been standard practice for many years, and no hazardous residues are left in foodstuffs properly treated.

Although acute illness due to short-term exposure to the quantities of pesticides present as residues in our food and water supply would not be expected to occur and has not occurred, the question of whether or not deleterious effects may result from long-continued ingestion of residues is more difficult to solve. A number of studies cited in more detail elsewhere in this review bear on this matter. Chronic feeding studies with experimental animals give information on tolerated and on toxic levels, as well as data on specific biochemical, physiologic, or anatomic abnormalities that can be expected to occur as a result of overdosage. Also, rats, for example, can be maintained on test dosages over their entire life span of approximately two years, while such lifetime studies with larger animals, including man, would not be feasible, due to the very long period of time required. With man, useful information may be obtained, as was done by Hayes *et al.* (1956), by giving larger doses over a period that is long but far less than a lifetime. This maneuver allows one to take advantage of the fact that any effects of pesticides on man would be expected to appear, on the average, the most promptly, most frequently, most diversely, and most severely among persons whose exposure has been long and intensive (*A.M.A. Committee on Pesticides* 1956). Use experience, accidental poisoning cases, and human volunteer studies also give information relative to the long-term hazard of residues.

b) Cases involving contamination during storage or shipment

Contamination of foodstuffs with pesticides can also occur during shipment.

In Britain, in 1956, 49 people became ill after eating bread made from endrin-contaminated flour (Davies and Lewis 1956). This flour became contaminated during shipment in a railway car in which endrin had previously been spilled. There were no fatalities in this group of cases.

Over 100 deaths in India during the spring of 1958 resulted from eating food accidentally contaminated with a pesticide during shipment (Karunakaran 1958). The board of inquiry found that there had been faulty storage of parathion in polyethylene containers, failure to pack these containers with absorbent materials, failure to provide adequate labelling, and a deliberate false description of the consignment in the waybills and invoices covering the shipment of the pesticide, since the product had been classified as "harmless".

In Singapore, in 1959, in an outbreak of parathion poisoning due to contaminated barley, 35 children and three adults became ill and nine children died (Kanagaratnam *et al.* 1960). Contamination of the barley apparently occurred during shipment, although the exact circumstances could not be determined. Study of the amounts of poison consumed indicated that younger children were more susceptible. Calculations suggested that the lethal dose of parathion was about 0.1 mg./kg. Only atropine was available for antidotal use in Singapore at this time.

It is disturbing to note, in connection with contamination during shipment, that similar incidents occur with the more toxic organic phosphorus compounds even in countries that actively enforce very strict regulations regarding labelling and shipping of poisonous materials. Although no cases involving contamination of foodstuffs are known to have taken place in the United States, a related accident did occur during 1961 in California (*California State Department of Public Health* 1961). A quantity of boys' denim trousers were shipped from the manufacturer to a retail store by a commercial freight line's motor van. The van also carried 11 other shipments, including 120 gallons of 50-percent Phosdrin emulsifiable concentrate in 72 containers. The truck driver noticed a puncture near the top in a five-gallon can of Phosdrin, and it was later determined that 1.13 gallons of Phosdrin emulsifiable concentrate had been lost. The consignee noted that one bale of the trousers was soiled and they were assigned for sale at a salvage store. Six boys who purchased and wore the contaminated trousers were poisoned, two of them quite severely. It requires little imagination to visualize the possible outcome if a foodstuff had become contaminated instead of the trousers in this incident.

c) Poisoning in crop-workers from residues

In addition to the importance of pesticide residues on food crops as potential hazards to human health by ingestion, residues on food crops have caused poisoning in agricultural workers from occupational exposure.

QUINBY and LEMMON (1958) reported 11 episodes of poisoning from contact with parathion residues involving more than 70 persons. Diagnosis was confirmed from anticholinesterase effect in seven episodes and from favorable response to atropine in nine outbreaks. The clinical picture in this type of poisoning, produced only by residues on the foliage of plants, was somewhat different from that produced in most cases by direct exposure during spraying or dusting. Residue poisoning has a more gradual onset and is usually a mild disease. Gastrointestinal symptoms usually predominate. Residue poisoning also differs epidemiologically from poisoning by direct exposure. In connection with residue poisoning, it has frequently happened that a large proportion of the persons exposed become sick; whereas, in poisoning after direct exposure to formulations it is unusual to have more than one or two cases among any group of workers. The crops involved in these episodes of poisoning by residues have included pears, apples, grapes, citrus fruits, and hops. The poisoned workers were engaged in picking, thinning, cultivating, or irrigating. Several of the outbreaks involved exposure to foliage or fruit sprayed not more than two days earlier. However, the age of residue involved ranged up to as much as 33 days. Absorption was thought to be by the dermal rather than by the respiratory route, on the basis of comparative exposure studies carried out under experimental conditions. Absorption of toxicant was favored by failure to wear protective clothing or by the persistent wearing of contaminated clothing. Although the influences predisposing toward the occurrence of residue poisoning are essentially unknown, certain weather conditions, including temperature and rainfall, may play a role in the etiology of the disease.

VII. Discussion

Much research has been done and is being continued in an attempt to evaluate the potential health hazard of pesticide residues in food and water. However, there are a number of facts, which have been reviewed in detail above, that indicate that these residues, as they actually occur in our food and water supply at the present time, do not pose a significant threat to human health.

In the United States, a safety factor of 100 is taken into account in setting the tolerance value for pesticide residues in food. An additional safety factor is included in the recommendations for application. Most food products which have been tested in this regard show pesticide residue content well below the tolerance level. Tests of complete meals for DDT content have shown a small degree of contamination. Use experience, based particularly on groups of individuals with long and intensive exposure to pesticides, indicates that these materials can be used safely. Available knowledge of modes of action of pesticidal chemicals makes it possible to look more effectively for incipient and minimal evidences of toxicity in occupationally exposed individuals and in the general population.

There can be no doubt that the very real hazard of certain insecticidal compounds requires appropriate precautions on the part of those using them. In many, if not most, cases of occupational poisoning, and in all known illnesses associated with consumption of foods bearing pesticide residues, careful study of the situation shows that one or more safety precautions were violated. Scientists who have studied the matter carefully agree that pesticides can be used safely provided recommended precautions are followed. Recommendations made on the basis of studies required under existing legislation would appear to exclude the possibility of characteristic toxic effects in the general population following ingestion of residues even for a lifetime. The safety factors are adequate to cover the quantitative differences known to occur in some instances between species or between different individuals of the same species. One cannot rule out the possibility of qualitative differences between species. That is why it is so important to maintain surveillance of workers who handle pesticide formulations in agriculture or public health work. Even with safety precautions, their exposure is far greater than that of the general population and any subtle or delayed effects would occur first and perhaps exclusively among these workers. Such studies should give warning of even the slightest danger to people in the general population exposed to traces of the same compounds.

It is reassuring to note that the effect of the various pesticides studied on animals, including man, seems to follow a normal dosage response curve, meaning that small amounts are probably harmless while larger doses may produce poisoning. Although a number of the common pesticides, including DDT, are stored in the body, this storage is not indefinitely cumulative. With tolerated doses of DDT, man approaches storage equilibrium in about one year and does not store any more of the compound regardless of how long the dosage is continued.

Continued research is neccessary to assure the safety of insecticidal chemicals. This is true both with respect to continued need for following long-term exposure to the older compounds and for detecting possible early effects of the newer materials.

Summary

The presence of any foreign chemical in the general food or water supply is a matter not to be dismissed lightly. The pesticides are poisons, of course, or they would not be useful in the control of insects, rodents, and other undesirable animals and plants. In considering the hazards of pesticide usage to human health, one should not overlook the fact that a number of health benefits have resulted, also.

That the general population ingests small amounts of the pesticidal chemicals remaining as residues on food has been viewed with alarm by a number of people. Data bearing on the toxic hazard due to pesticide residues are available from studies in which the amount of residue present in various foods has been determined and from animal toxicity experiments, and may be available from use experience, human volunteer studies, and cases of poisoning due to accident or suicide attempt. Studies are more nearly complete for DDT than for any other pesticidal chemical. Results from each of these types of study seem to indicate that no significant hazard to human health exists from pesticide residues as they now occur in our diet in the United States. However, the majority of pesticides have been little studied in man, and — even for the better known ones — there is no way to exclude the possibility that subtle dangers remain to be discovered.

No known cases of illness in the United States have resulted from insecticide residues on food when formulations have been used according to directions. The same is true in the United Kingdom. However, there have been several instances in which insecticides used quite improperly on foods have led to acute poisoning soon after ingestion. Contamination of foodstuffs with pesticides can also occur during shipment or storage.

In the United States, a safety factor of 100 is taken into account in setting the tolerance level allowed for pesticide residues in food. An additional safety factor is included in the recommendations for application.

It is reassuring to note that the effect of the various pesticides studied on animals, including man, seems to follow a normal dosage response curve, meaning that small amounts are probably harmless while larger doses may produce poisoning. While a number of the common pesticides, including DDT, are stored in the body, this storage is not indefinitely cumulative. With tolerated doses of DDT, man approaches storage equilibrium in about one year and does not store any more of the compound regardless of how long the dosage is continued at the same rate.

Continued research is necessary to assure the safe use of insecticidal chemicals. This is true both with respect to continued need for following long-term exposure to the older compounds and for detecting possible early effects of the newer materials.

Résumé *

La présence de tout produit chimique étranger dans les aliments ou les eaux est un sujet qui doit être traité avec circonspection. Les pesticides sont des poisons; s'ils ne l'étaient pas, ils ne seraient pas utiles pour détruire les insectes, les rongeurs et autres organismes animaux et végétaux indésirables. En examinant les dangers inhérents à l'usage des pesticides du point de vue de la santé publique, il ne faut cependant pas négliger les bienfaits qui leur reviennent.

Un certain nombre de personnes se sont alarmées en apprenant que la population absorbe de petites quantités de résidus de pesticides qui per-

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* Traduit par S. Dormal.

sistent dans les denrées alimentaires. Les données dont on dispose sur les risques d'intoxication par les résidus de pesticides proviennent d'études qui ont porté sur la détermination des résidus dans différents aliments et d'expérimentations toxicologiques sur animaux ainsi que, éventuellement, de l'expérience relative à l'usage des produits, d'études pratiquées sur volontaires et de cas d'empoisonnements résultant d'accidents ou de tentatives de suicides. Les études sur le DDT sont plus complètes que celles qui se rapportent à tout autre produit. Les résultats de chaque type d'études semblent indiquer que les résidus de pesticides, tels qu'ils se présentent aux Etats-Unis dans notre alimentation, ne peuvent être cause de danger réel pour la santé publique. Cependant, la plupart des pesticides ont été peu étudiés sur l'homme et — même pour ceux qui sont les mieux connus — il n'y a pas lieu d'exclure la possibilité de leur découvrir des effets insidieux.

Aux Etats-Unis, aucun cas connu de maladie n'a pu être attribué aux résidus de pesticides dans les aliments à la suite d'un usage des formulations conforme aux directives. Il en est de même pour le Royaume-Uni. Cependant, il existe de nombreux cas où des insecticides utilisés improprement sur des aliments ont entraîné l'intoxication aiguë peu après l'ingestion. La contamination des denrées alimentaires par les pesticides peut aussi survenir lors du transport ou de la conservation.

Aux Etats-Unis, un facteur de sécurité de 100 est pris en considération lors de la fixation des limites de tolérance pour les résidus de pesticides dans les aliments. Un facteur de sécurité supplémentaire est inclus dans les recommandations relatives à l'application des produits.

Il est rassurant de constater que l'effet des différents pesticides étudiés sur animaux, y compris l'homme, semble suivre une courbe normale dose-réponse impliquant que les petites doses sont vraisemblablement inoffensives tandis que les doses plus élevées sont susceptibles d'entraîner l'empoisonnement. Bien que un certain nombre de pesticides d'usage courant, comprenant le DDT, s'accumulent dans l'organisme, cette accumulation ne croît pas indéfiniment. Avec les doses tolérées de DDT, le taux d'accumulation chez l'homme tend vers un équilibre endéans un an environ et, quelle que soit la durée de l'absorption, il ne se modifie plus si la quantité de pesticide absorbée reste constante.

Il est nécessaire de poursuivre les recherches en vue d'assurer un usage inoffensif des pesticides. Ce besoin vise autant l'étude des effets de l'exposition à long terme pour les pesticides connus que celle de la détection des effets immédiats possibles des nouveaux produits.

Zusammenfassung *

Das Vorkommen irgend eines chemischen Fremdstoffes in den üblichen Lebensmitteln und im Wasser ist eine Sache, die nicht nebenbei abgehandelt werden darf. Die Schädlingsbekämpfungsmittel sind natürlich Gifte, sie könnten sonst nicht zur Bekämpfung von Insekten, Nagetieren oder anderen unerwünschten Tieren und Pflanzen dienen. Wenn man die Gefahren der

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* Übersetzt von O. R. KLIMMER.

Verwendung von Schädlingsbekämpfungsmitteln für die menschliche Gesundheit erwägt, sollte man die Tatsache nicht vergessen, daß sie auch eine Anzahl von gesundheitlichen Vorteilen mit sich brachten.

Die Tatsache, daß die Gesamtbevölkerung kleine Mengen von Schädlingsbekämpfungsmitteln, die als Rückstände in Lebensmitteln verbleiben, zu sich nimmt, hat auf eine Anzahl Leute alarmierend gewirkt. Unterlagen bezüglich der Giftgefahren durch Schädlingsbekämpfungsmittel-Rückstände lassen sich aus Untersuchungen, in denen Rückstandsmengen in verschiedenen Lebensmitteln bestimmt wurden, sowie aus Toxicitätsversuchen an Tieren gewinnen, sie können aber auch aus Erfahrungen bei der Anwendung, aus Versuchen an freiwilligen Versuchspersonen und aus Vergiftungsfällen durch Unfälle und Selbstmordversuche stammen. Mit DDT sind umfassendere Untersuchungen als mit irgend einem anderen chemischen Schädlingsbekämpfungsmittel durchgeführt worden. Die Ergebnisse aus all diesen verschiedenen Untersuchungen scheinen darauf hinzudeuten, daß die Schädlingsbekämpfungsmittel-Rückstände, wie sie jetzt in unseren Lebensmitteln in den USA vorkommen, für die menschliche Gesundheit keine nachweisbare Gefahr darstellen. Jedoch ist die Mehrzahl der Schädlingsbekämpfungsmittel am Menschen wenig untersucht worden und — selbst bei den bekannteren — kann man die Möglichkeit nicht ausschließen, daß latente Gefahren nicht eines Tages doch noch entdeckt werden.

In den USA sind Erkrankungsfälle als Folge von Insecticidrückständen in Lebensmitteln nicht bekannt geworden, wenn die Formulierungen gemäß den Vorschriften angewendet wurden. Dasselbe gilt für England. Jedoch gibt es einige Fälle, in denen Insecticide bei Nahrungsmitteln ganz unsachgemäß zur Anwendung kamen und bald nach dem Verzehr zu akuten Vergiftungserscheinungen geführt haben. Die Verunreinigung der Lebensmittel durch Schädlingsbekämpfungsmittel kann auch während des Transportes und der Lagerung erfolgen.

In den USA wird ein Sicherheitsfaktor von 100 bei der Festsetzung der Toleranzwerte für Schädlingsbekämpfungsmittel-Rückstände in Lebensmitteln in Betracht gezogen. Ein zusätzlicher Sicherheitsfaktor ist bei den Empfehlungen für die Anwendung dieser Stoffe berücksichtigt.

Die Feststellung, daß die Wirkung verschiedener Schädlingsbekämpfungsmittel, die an Mensch und Tier untersucht wurden, einer normalen Dosis-Wirkungskurve folgt, ist beruhigend. Das bedeutet, daß kleine Mengen wahrscheinlich harmlos sind, während größere eine Vergiftung hervorrufen können. Während die Anzahl der üblichen Schädlingsbekämpfungsmittel, einschließlich DDT, im Körper abgelagert wird, ist diese Ablagerung nicht unbegrenzt kumulativ. Bei verträglichen Dosen erreicht der Mensch ein Speicherungsgleichgewicht nach etwa 1 Jahr und speichert dann kein weiteres DDT mehr, gleichgültig wie lange die Zufuhr in gleicher Höhe fortgesetzt wird.

Weitere Forschungsarbeit ist notwendig, damit die Unbedenklichkeit der Verwendung chemischer Insecticide bestätigt werden kann. Dies gilt sowohl in Hinblick auf die bleibende Notwendigkeit, weitere chronische Versuche mit den älteren Stoffen durchzuführen, als auch mögliche Frühwirkungen neuerer Stoffe aufzudecken.

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DURSBAN PREMISES APPLICATIONS AND THEIR EFFECT ON THE CHOLINESTERASE LEVELS OF SPRAYMEN

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Small

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Dursban[5], o,o-diethyl o-3,5,6-trichloro-2-pyridyl phosphorothioate, has been demonstrated to be effective against numerous mosquito species in diverse habitats. Ludwig and McNeil (1966) reported a high degree of biological activity against larvae and adults of *Aedes aegypti* and *Aedes sollicitans* and larvae of *Culex quinquefasciatus* when it was applied as a granular formulation, emulsion, or thermal fog. Sjogren and Mulla (1968) obtained good results with Dursban emulsifiable concentrate applied by the drip method for the control of mosquitoes in sewage ponds. Brooks *et al.* (1967) demonstrated 19 weeks of effective kills of *Ae. aegypti* larvae in cans and tires with emulsifiable Dursban. Mathis and Schoof (1968) reported Dursban residues on clay surfaces to be highly effective against *Anopheles quadrimaculatus*.

Because of its high level of effectiveness as a mosquito larvicide, field trials with Dursban against *Ae. aegypti* were started in Perrine, Florida, in 1966. However, the adverse effect of these treatments upon the cholinesterase level of the spraymen led to the termination of the study within 2 weeks after spraying was begun. Since the application rates and the amount of exposure in these tests were extreme, a second study was conducted in 1967. These results indicated no adverse effects on the four spraymen, but the limited exposure period and amount of Dursban applied necessitated a further and more intensive evaluation designed primarily to measure the hazard of the compound to exposed spray personnel. Consequently, in 1968 a closely monitored field test was set up to determine what effect Dursban would exert on spraymen applying treatment at the rates normally encountered on operational programs for eradication of *Ae. aegypti*. The present paper describes the three studies with the major reference given to the 1968 investigations.

METHODS

Insecticide Treatment Procedures—1966 Test. The area sprayed consisted of premises and vacant lots normally encountered in a subtropical, lower socioeconomic neighborhood. Lush and heavy vegetative growth was common. Dursban as a 0.5 percent emulsion was applied by means of power spray equipment using an application pressure of 250 pounds per square inch. All types of premises and vacant lots were treated, some of which contained an extremely heavy plant growth. The high pressure resulted in micronization and considerable splashback of the finished spray. The average number of gallons applied per premises was 29.1. Five spraymen and one foreman were involved in the 9-day spraying period.

1967 Test. This test was carried on

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[5] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health, Education, and Welfare.

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in an area which had a low *Ae. aegypti* positive container index. Dursban was applied as a 0.25 percent suspension and as a 0.5 percent emulsion. This test was run for only 5 days during which 177 gallons of suspension and 145 gallons of emulsion were applied to two 9-block areas containing 182 premises. The average number of gallons sprayed per premises was 0.5. Four spraymen, one foreman, and two controls were involved.

1968 TEST. These studies were conducted in an area similar to that used for the 1966 tests with the exception that vacant lots, premises heavily overgrown with vegetation, and large piles of containers were eliminated from the spray schedule. Dursban was applied as a 0.5 percent suspension or emulsion with a separate crew spraying each type of formulation. Treatment was made with truck-mounted power equipment with the working spray pressure at 125 pounds per square inch. A pistol-type spray gun was used in preference to the orchard type employed in 1966. The pistol-type made it possible to produce a coarse cone spray without having to adjust through a fine particulate spray pattern as was necessary with the orchard type unit. The average number of gallons applied per premises was 4.6.

Individual containers were treated and blanket spraying avoided. The seven spraymen and hosemen were instructed to stay upwind of the spray gun wherever possible to minimize exposure to spray drift. They were cautioned to spray only in open areas and to avoid heavy vegetative undergrowth where they might be more subject to inhalation of airborne spray particles. Clean, uncontaminated clothing was provided daily. Protective masks and gloves were worn by men that handled the concentrate when mixing the formulation in the sprayers. The foreman was cautioned to have as little contact with the spray as possible. This individual and three persons who would have no contact with cholinesterase inhibiting compounds during the test period served as controls.

CHOLINESTERASE DETERMINATION. Cholinesterase measurements were made by the pH stat method as described by Nabb and Whitfield (1967). In 1966 and 1967 the weekly venous blood samples were drawn locally, packed in ice, and shipped to the NCDC Pesticides Program, Toxicology Section, for cholinesterase value determinations. In 1968 all cholinesterase values were determined locally by the Pesticides Research Laboratory. Determinations were made on the day following the withdrawal of the blood samples from the spraymen and were immediately available to us. Values reported (Table 1) are expressed in micromoles of acetylcholine hydrolized per minute per milliliter of plasma or packed red blood cells.

In each test it was decided to terminate the exposure of any individual if a depression of 50 percent of his normal baseline activity was observed. This level was chosen as the criterion, since symptoms of organophosphate poisoning rarely appear until this level is reached and, therefore, the chances of deleterious effects are still negligible (Goltz and Shaffer, 1960).

RESULTS

Since the 1966 study was primarily done to test the larvicidal activity of Dursban against *Ae. aegypti* and cholinesterase activity in the spraymen was a secondary consideration, the study was not designed to monitor closely the output of the individual sprayman. However, it was readily evident that three spraymen showed a marked depression of their plasma cholinesterase, the values ranging from 68 to 82 percent reduction of the normal pretreatment exposure levels. Two other spraymen for whom pretreatment values were not available showed 52 to 56 percent reduction in their plasma values over a 9-day spraying period. Because of the significant cholinesterase inhibition that occurred in these spraymen, the study was discontinued after 2 weeks of spraying.

The data for the 1967 application indicated that none of the four spraymen showed any significant difference between the pretreatment and the posttreatment cholinesterase value determined on three separate occasions during the 1 week of spraying. However, the plasma cholinesterase level for the foreman did fall from a level of 4.5 to 2.1 over the 7-day period. Since this individual was not involved in actual spraying, the reason for this depression was not apparent. Although no preexposure level was available for the second foreman, his plasma values for the same period ranged between 4.5 and 4.8. The small sample and the limited time and amount of exposure were such that the above findings on the spraymen permit only a limited interpretation. These data required validation and confirmation

Table 1.—Cholinesterase levels[1] in spraymen applying known amounts of 0.5 percent Dursban as a larvicide for *Aedes aegypti* control (1968 test).

			Exposure			Postexposure	
		Preexposure[2]	April 25	May 2	May 6	May 23	June 12
Suspension							
Sprayman 1	RBC	14.8	16.0	16.6	18.8	13.2	16.7
	Plasma	4.6	1.1	0.4	1.0	2.4	3.2
	Gal. sprayed[3]	0	141	301	65	20	0
Sprayman 2	RBC	12.3	16.9	13.2	19.2	12.3	13.9
	Plasma	4.0	1.9	1.1	1.2	2.8	4.0
	Gal. sprayed[3]	0	109	260	85	10	0
Sprayman 3	RBC	14.0	16.5	..	22.5	..	16.5
	Plasma	4.7	5.0	..	2.2	..	4.9
	Gal. sprayed[3]	0	90	..	355	..	32
Emulsion							
Sprayman 4	RBC	15.3	18.0	17.0	21.0	13.0	16.2
	Plasma	5.0	3.5	2.4	2.3	3.7	5.5
	Gal. sprayed[3]	0	70	165	35	20	0
Sprayman 5	RBC	12.2	15.0	16.0	..	13.4	13.9
	Plasma	4.3	3.8	4.0	..	4.4	5.5
	Gal. sprayed[3]	0	55	47	..	115	0
Sprayman 6	RBC	15.0	17.9	15.4	..	..	15.2
	Plasma	5.6	4.5	3.3	..	..	4.9
	Gal. sprayed[3]	0	59	210	..	..	110
Sprayman 7	RBC	12.8	13.0	14.8	..	..	14.1
	Plasma	5.2	5.8	4.4	..	..	5.3
	Gal. sprayed[3]	0	51	65	..	..	85
Foreman	RBC	15.9	14.7	18.0	..	13.8	
	Plasma	4.3	4.9	4.3	..	4.0	
Control 1	RBC	15.1	16.9	14.0	..	12.0	
	Plasma	3.7	3.2	3.8	..	3.9	
Control 2	RBC	10.5	13.1	13.0	..	11.2	
	Plasma	4.2	4.0	4.0	..	4.4	
Control 3	RBC	11.1	14.1	13.6	..	12.0	
	Plasma	5.6	5.9	5.7	..	6.1	

[1] Values reported are expressed in micromoles acetylcholine hydrolized/minute/ml. plasma or packed red blood cells.

[2] Average for analyses done prior to commencement of spraying.

[3] Gallons of finished spray applied between consecutive blood analysis dates.

in a study over a much longer period during which a much higher degree of exposure would be involved.

In the 1968 study five of the seven spraymen showed more than 50 percent reduction of plasma cholinesterase values within 2 weeks after treatment was begun, thus necessitating a termination of the test. The data on the exposure and the cholinesterase levels for the spray personnel, foreman, and controls are shown in Table 1. Analyses of the data for these individuals show that the quantity of Dursban sprayed proportionately inhibits plasma cholinesterase. No clinical manifestations of organophosphate poisoning were noted in any of the spraymen although in the case of Sprayman No. 1, there was over a 90 percent decrease in his plasma enzyme level on May 2 as compared to his pre-exposure level. No inhibition of red cell cholinesterase activity was observed at any time.

A cholinesterase level for an individual may be considered abnormal if it shows excessive deviation from his own normal values, or if it falls outside the range of values observed in a normal population. There is a reasonably large range in plasma (23 percent) and red cell (25 percent) cholinesterase determinations taken periodically from the same normal, unexposed individual (Ganelin *et al.*, 1964). Nabb and Whitfield (1967) found a range of 3.6–6.8 μM of acetylcholine hydrolyzed per minute per milliliter of plasma. Judged either on the basis of their individual normal values, or on the basis of the values for a normal population, all of the spraymen, with the exception of numbers 5 and 7, exceeded the expected deviation for plasma cholinesterase activity, although the values for the foreman and the controls remained within the expected range.

DISCUSSION

In general, the expected results of exposure to most organophosphate insecticides, as measured by blood cholinesterase activity, is the initial depression of plasma cholinesterase followed by a decrease of red blood cell cholinesterase (Goltz and Shaffer, 1960). The rate and extent of the decrease in activity relate to the amount of organophosphate absorbed. Dursban did produce the expected effect that the amount and rate of depression were related to the gallonage sprayed. The question of whether or not a decrease in red cell cholinesterase would have been observed if a longer exposure period at the same average daily rate had been used is not answerable from the data obtained.

However, in laboratory tests (Gaines, T. B., unpublished data) the dosages of Dursban (e.g., 1.0 mg./kg.) that decreased the plasma cholinesterase levels in rats usually exerted a similar but lesser degree of lowering of the red blood cell cholinesterase. A critical dosage that had only a slight depressive effect on the plasma cholinesterase was associated with a small rise in the red blood cell cholinesterase. In the 1968 spray trial, a similar slight increase in red cell cholinesterase was observed in the men during the exposure period, and it persisted irregularly into the postexposure period. No increase was seen during the 1966 study. The increase in red cell cholinesterase activity associated with dosages just adequate to inhibit plasma enzyme to some degree is not fully understood, but it has been observed occasionally in animals in connection with some other compounds. It is thought to represent an overcompensation without clinical significance.

These findings for Dursban when used as a larvicide treatment under outdoor conditions were not anticipated when the original study commenced. Although Dursban, in common with all organophosphate insecticides, inhibits cholinesterase (Gray, 1965) the oral LD-50 value for it with female rats is 82 (Gaines, 1969) to 135 mg./kg., a value range in which a compound is generally considered as only moderately toxic. While the results of the initial tests were at first thought to be due to the high gallonage applied per premises, the amount of material applied during the 1968 study was at a level considered to be in the range of the normal amount of spray applied in the *Ae. aegypti* operational program. Although the results for 1967 showed no effect on the cholinesterase of the spraymen, the gallonage applied was below that normally sprayed on operational programs and, consequently, the primary interpretation of the suitability of Dursban as a premises larvicide against *Ae. aegypti* must be based on the data obtained in 1968. The findings for the last test confirm the results obtained from the original work in 1966 even though there was an 84 percent reduction in the average amount of Dursban spray applied per premises. The application of a suspension formulation apparently had no advantage over the use of an emulsion nor was there any obvious beneficial effect of reducing the spray pressure from 250 to 125 pounds per square inch.

Inasmuch as a compound that produces a measurable decrease in plasma or red blood cell cholinesterase level in spraymen is not considered to be a suitable material for use in public health work in the absence of an epidemic or the immediate threat of one, the conclusion is that Dursban emulsion or suspension formulations are not acceptable for use as a premises larvicide treatment in the control of *Ae. aegypti* under the conditions now present in the United States. It is conceivable that Dursban could be used as a granular formulation, at a much lower dosage as described for a nonresidual type of larviciding or at an intermittent or less frequent interval without any effect on cholinesterase levels. The findings also suggest that the use of Dursban in any operation similar to that described previously might produce a depression of cholinesterase levels. Therefore, on all such control programs, careful monitoring of the cholinesterase levels of spraymen should be included in the operation.

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